QUANTITATIVE ASPECTS OF IODINE METABOLISM IN MAN*

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I. INTRODUCTION

During the past fifteen years three developments have enormously facilitated the quantitative study of iodine metabolism and thyroid function. The first of these has been the production of radioactive isotopes of iodine. The second has been the gradual perfection of accurate methods for the separation, identification and chemical determination of the minute amounts of iodine-containing compounds encountered in biological materials. The third has been the discovery of pharmacological agents which, by interfering with certain specific steps in the manufacture of thyroid hormone, make possible the biochemical dissection of thyroid function in living animals. Much of the new information which has accrued from the use of these physical, chemical, and pharmacological tools has already been reviewed by others. Excellent discussions of the steps in the synthesis of thyroxine by the thyroid gland (70, 71), the mechanism of action of goitrogenic drugs (7, 8, 27, 133), the occurrence of thyroid-inhibiting compounds in food (58), and the mode of action of the thyroid hormone in tissues (12) have recently been published. In addition, several authors have presented well-balanced appraisals of the clinical value of the various methods currently available for the diagnosis (81, 107, 137, 175), and treatment (7, 32, 106, 107, 108, 110, 177) of thyroid disease. These topics will be mentioned here only briefly or not at all.

The present review is an attempt to describe, as precisely as present information will permit, all the factors which influence iodine metabolism and hence the activity of the thyroid gland. Although the results of animal experiments will sometimes be mentioned, most of the discussion will be concerned with man. The scope of this review is further restricted by deliberate neglect of the mechanism of hormone secretion (42), and the metabolism of synthetic iodine-containing compounds which are foreign to the body. Besides these restrictions, the author must confess that his review of the literature has been highly selective, rather than comprehensive, and that he may have inadvertently overlooked important papers. Fortunately much of the recent work on iodine metabolism, including information derived from animal experiments, has been very well

summarized by Albert (1), and his extensive bibliography contains a number of pertinent references not included in the present paper. It is hoped that in spite of these deficiencies the review will be useful as an illustration of how the direct measurement of a few variables can be combined with simple mathematical deductions so as to provide a reasonably complete and detailed *quantitative* description of the metabolic pathways followed by a chemical element in man.

II. QUANTITATIVE METHODS AVAILABLE FOR THE STUDY OF IODINE METABOLISM IN MAN

A. *Chemical Methods:* The ability of traces of the iodide ion to catalyze the reaction between ceric salts and arsenious acid was first described by Sandell and Kolthoff (170). Chaney adapted this reaction to the analysis of iodine in biological materials (25), and many exquisitely sensitive modifications of the Chaney method are now in widespread use (11, 22, 31, 97, 186). With this method it is possible to detect quantities of iodine as small as 0.005 microgm. Somewhat larger quantities may be measured with considerable precision so that one or two ml. of plasma or serum suffice for a single analysis. The analytical technique is also far less time-consuming and far more objective than previous methods. However, except for occasional analyses of iodine in lymph, cerebrospinal fluid or edema fluid, most analysts have restricted themselves to serum, urine or samples of the thyroid gland removed at operation or autopsy. Few recent studies have dealt with the iodine content of food, feces or sweat.

B. *Radioactive Iodine:* The advantages of radioactive iodine as a tool for the investigation of iodine metabolism and thyroid function have been adequately described by others (80, 83, 141, 149, 150, 175) and need not be reiterated here. The isotope I^{131} with a half life of 8.0 days is made available as iodide from the uranium pile at Oak Ridge to investigators with proper facilities for its safe handling. Since it is available practically uncontaminated with inert iodine ("carrier-free") it can be given either by mouth or by vein without causing any detectable increase in the total quantity of iodine in the body. It can thus be used to measure the dynamic behavior of iodide without upsetting normal equilibrium. Much of the usefulness of radioactive iodine lies in the possibility of measuring it not only in samples of body fluids and urine, but also *in vivo* as it accumulates in the thyroid gland. A discussion of the precautions required for accurate measurements of radioactivity in the thyroid gland lies outside the scope of this paper. Suffice it to say that due consideration must be given to the elimination of radiation coming from other parts of the body, to the effect of the surrounding neck tissues on radiation emanating from the gland, and to the geometrical relationship between the gland itself and the counting apparatus (82, 120, 127).

The techniques of butanol extraction and chromatographic separation have been used to good advantage in the separation and identification of the various compounds into which radioactive iodine becomes incorporated during its sojourn in the body. These preparative techniques are often facilitated by the addition of comparatively large quantities of the iodine-containing compounds

under scrutiny, the added "carrier" compounds containing only I^{127}. When carriers are used, the conditions of extraction and purification must preclude exchange reactions between the stable and radioactive isotopes (89, 112).

Two practical considerations limit the quantity of radioactivity which can be administered for physiological studies in the human. While in certain patients with hyperthyroidism or thyroid carcinoma amounts of radioactive iodine sufficiently large to damage the thyroid gland are purposely administered, it should be obvious that under these circumstances the fate of the radioactive iodine cannot provide reliable information about the *normal* physiology of the thyroid gland. During the first few days after the administration of such a "therapeutic" dose, the damaged thyroid parenchyma may occasionally permit large quantities of preformed hormone to leak out into the blood stream (155, 163). Not only will this cause an elevation in the concentration of serum protein-bound iodine, but there is also evidence that some of the material thus gaining access to the circulation is actually thyroglobulin (152, 193) which under normal circumstances is never secreted by the thyroid gland (91). Later, the diminution in thyroid activity caused by radiation damage will continue to upset the equilibrium which was presumably present at the time of administration of the radioactive iodine. For physiological studies it is therefore necessary to limit the dose to one which will surely not produce any detectable alteration in thyroid function. Although estimates vary, it seems probable that single doses of 100 microcuries or less will comply with this requirement.

The second limitation on the quantity of radioactive iodine which may legitimately be administered to man can neither be easily defined nor lightly dismissed (20, 32, 125). It is well recognized that exposure of a tissue to ionizing radiation may eventually produce malignant degeneration, often years after the initial exposure (45). While no instances of carcinogenesis due to the administration of radioactive iodine to man have as yet been reported, the production of thyroid carcinomas in rats given large doses of radioactive iodine has been clearly demonstrated by Goldberg and Chaikoff (50). Until enough time has elapsed to permit a final evaluation of the late effects of radioactive iodine in humans, every investigator is morally obliged to limit the quantity of radioactive iodine administered for physiological studies to the smallest amount which will suffice for the purpose at hand. This is of particular importance when repeated administration of tracer doses to the same individual is contemplated, since there is evidence that the effects of exposure to radiation are cumulative (20, 118).

These restrictions on the quantity of radioactive iodine which may be legitimately employed for physiological studies limit the time after a tracer dose during which reliable measurements of radioactivity can be made. In long-term studies not only is radioactive iodine lost through various excretory channels, but also the physical decay inexorably reduces the quantity of radioactive iodine remaining in the body. Thus, even if there were no excretion of iodine, a study involving serial measurements for a period of a month after a single tracer dose would require the administration of about fifteen times as much radioactive

iodine as would suffice for a brief study of comparable precision. Fortunately, the development of more sensitive recording devices such as the scintillation counter has considerably extended the time during which useful measurements can be made after the administration of a tracer dose of radioactive iodine (5, 19).

A sharp distinction must be drawn between the kind of information obtained with tracer doses of radioactive iodine and the kind of information obtained with chemical methods of analysis. Tracer studies are ideal for the measurement of the *proportion* of the iodine in the body which follows a particular metabolic pathway and for the study of the *rate of turnover* of iodine within the various compartments. By themselves, however, tracer studies cannot indicate the actual amounts of iodine which are being metabolized. Chemical methods on the other hand give valuable information concerning the *quantities* of iodine with which the body deals, but by themselves provide no more than a dim outline of the dynamics of iodine metabolism. Only by the combined use of radioactive tracers and of chemical methods can a clear picture of the over-all metabolism of iodine be obtained.

III. NORMAL PATHWAYS OF IODINE METABOLISM

A. *General Description:* In the interests of clarity it is well to begin with a simple qualitative description of the various routes normally traversed by iodine entering the body. These routes are diagrammatically portrayed in Figure 1.

Iodide ingested with food and water is rapidly absorbed from the gastrointestinal tract into the blood stream. While some absorption may occur from the stomach (78), the majority is absorbed by the intestine. If administered as free iodine or iodate, reduction to iodide must occur in the intestine before absorption can take place (30). From the blood stream the iodide rapidly diffuses into the extracellular fluid of the tissues, its distribution for the most part being very similar to that of chloride (199). Although largely excluded from most cells, the iodide ion freely traverses the red blood cell membrane. The concentration of iodide in the water of red blood cells is approximately equal to its concentration in the water of plasma, so that a given volume of red blood cells contains about 65 per cent as much iodide as an equal volume of plasma (119, 144, 157). Distribution equilibrium between red blood cells and plasma is attained with great rapidity (119, 159).

In certain important respects, however, the distribution of the iodide ion differs significantly from the distribution of the chloride ion, particularly when the concentration of iodide is small. The most obvious of these differences is the accumulation of the iodide ion in the thyroid gland (see Section X, C). In addition, the concentration of iodide in salivary and gastric secretions far exceeds its concentration in extracellular fluid (119, 99). The thirty-fold increase in concentration achieved by the salivary and gastric glands is of the same order of magnitude as the concentration gradient which can be maintained by the normal thyroid gland. This similarity is particularly interesting since phylogenetically and embryologically the thyroid gland is derived from the anterior

portion of the primitive gut (51). It would be interesting to determine whether thyrotropic hormone increases the ability of the salivary glands and gastric mucosa to concentrate the iodide ion as it increases the concentrating capacity of the thyroid gland. As pointed out by Myant and his co-workers (119), the very high concentrations of iodide in saliva and gastric juice may account in part for the fact that the iodide ion has a larger volume of distribution than the chloride ion.

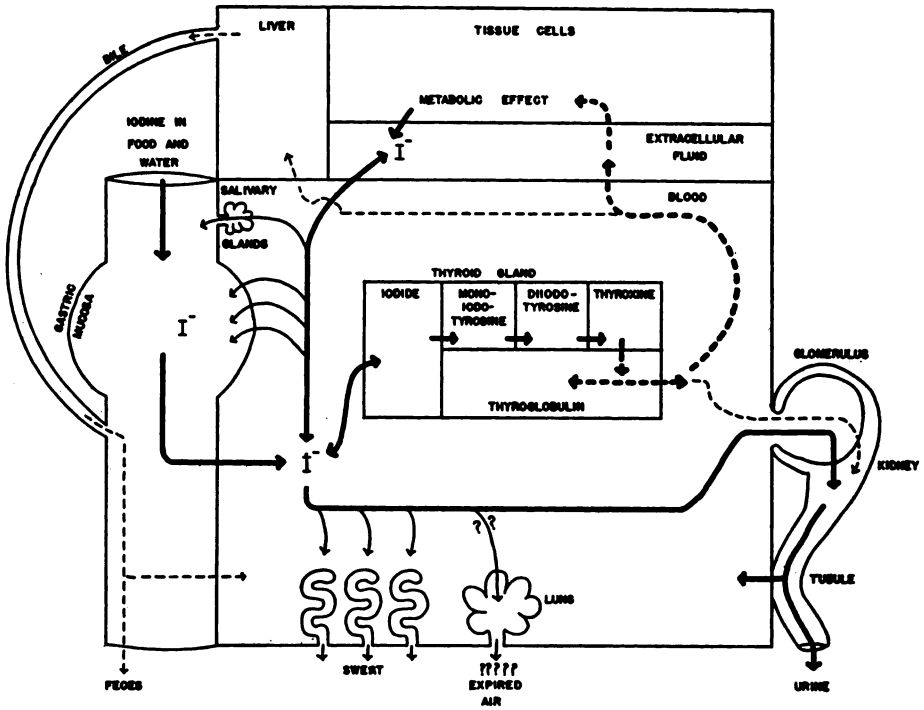

FIG. 1. A diagrammatic portrayal of iodine metabolism. Major pathways are represented by thick arrows, minor pathways by thin arrows. Solid lines designate inorganic, and broken lines organic iodine. It is obvious that the complex scheme depicted in this figure must be considerably simplified for convenient mathematical description.

Although small quantities of iodide may be lost from the body in sweat, feces and possibly expired air, the vast majority of the iodide in extracellular fluid eventually follows one of two pathways. It is either trapped by the thyroid gland to be manufactured into thyroid hormone, or excreted by the kidney. The thyroid gland and the kidney are therefore in competition with each other for the available supply of iodide. In the kidney the iodide is filtered through the glomeruli and is then partially reabsorbed by the tubules but to a far smaller extent than is chloride, at least in the human (see Section VI, A).

Once trapped by the thyroid, iodide is rapidly converted to organic iodine by oxidation and combination with tyrosine. In successive reactions monoiodotyro-

sine, diiodotyrosine and thyroxine are synthesized, and the completed hormone is pooled with the preformed hormone already stored in the gland as thyroglobulin.[1] Under the controlling influence of the thyrotropic hormone of the anterior pituitary gland the thyroid hormone is secreted into the blood stream in whatever amount is needed to maintain a reasonably constant plasma concentration. Although the thyroid hormone may leave the gland as free thyroxine (90), upon entering the blood stream it becomes rapidly, firmly and almost completely bound to the plasma proteins. Several studies with butanol extraction and chromatographic separation have conclusively demonstrated that practically all of the iodine bound to the plasma proteins is present as thyroxine (64, 88, 161, 188).

Unlike iodide, appreciable amounts of thyroxine do not enter the red blood cells (157). However, protein-bound iodine, presumably thyroxine or a derivative of thyroxine, has been demonstrated within liver cells (23, 109) and probably exists within the cells of most, if not all, tissues. By reactions as yet unknown, the thyroxine iodine in the tissues is reduced to inorganic iodide which once again enters the general pool of iodide in extracellular fluid. There are thus two sources of iodide ion within the body: absorption from the gastro-intestinal tract, and breakdown of thyroid hormone in the tissues.

Although the majority of the hormonal iodine probably follows the pathway just described, a portion may be removed from the blood stream by the liver and secreted with the bile into the intestine. Some of this may be reabsorbed, but a portion may be lost to the body in the feces. Finally, traces of thyroxine and diiodotyrosine have been demonstrated in the urine with chromatographic techniques.

B. *Simplifying Assumptions:* For the mathematical analysis of iodine metabolism it is convenient to introduce certain simplifying assumptions concerning the rather complex series of events described above. Although simplification can be achieved without doing serious violence to the major facts of iodine metabolism, it must be clearly recognized that the resulting scheme will be to a certain extent abstract and artificial. The employment of such an abstract model for a complicated biological system is warranted only if each assumption is explicitly stated and justified. This will be done in the following paragraphs.

1. *Compartments:* It will be assumed that all of the iodine in the body is divided into three separate compartments.

(a) *Inorganic Iodide:* This compartment contains all of the inorganic iodide in the body including the iodide in the secretions of the alimentary tract, the iodide in red blood cells, and the iodide which is present in the thyroid gland and which has not yet been incorporated into protein. Although Oddie (126) has chosen to treat the inorganic iodide in the thyroid gland as a separate compartment, there seems to be no compelling reason to do so, since there is good evidence that iodide ions within the thyroid are freely exchangeable with the

[1] Note that in Figure 1 the transformation of iodide to thyroxine is depicted as a "one-way street". The reviewer once suggested that the thyroid gland might be capable of degrading exogenous hormone to inorganic iodide (158). He now believes, in complete agreement with Greer (59), that this is highly improbable.

iodide ions in the blood stream. One practical difficulty does arise from inclusion of the iodide within the thyroid gland as a segment of the general iodide compartment. During studies with radioactive iodine, radiation emanating from iodide ions within the thyroid will necessarily be measured together with radiation from iodine which has already become organically bound, and may therefore erroneously be ascribed to the compartment of organic iodine in the thyroid gland. Usually the transformation of inorganic to organic iodine is so rapid that this error will be negligible. However, if conversion to organic iodine is blocked, due allowance must be made for the presence in the thyroid gland of a considerable amount of radioactivity which properly belongs to the iodide compartment.

(b) *Organic Iodine in the Thyroid Gland:* To this compartment are assigned all of the organic compounds of iodine which occur in the thyroid. No distinction is made between iodine present as monoiodotyrosine, diiodotyrosine, thyroxine, or any unidentified compounds which may also be present (64, 160, 189). Disregard of the separate molecular species actually present is justified by the impossibility of measuring the transformation of one to another in the intact human being.

(c) *Organic Iodine in the Blood and Extrathyroidal Tissues:* This compartment contains all of the thyroid hormone outside of the thyroid gland including any thyroxine which is undergoing enterohepatic circulation.

It should be obvious that these so-called compartments do not exist within the body as actual physical entities with clearly defined boundaries, but are merely convenient abstractions. Within each compartment the iodine is assumed to be uniformly distributed. If the actual concentration of iodine in one region of the body is relatively high, that region will represent a relatively large portion of the compartment. This may lead to occasional difficulties in interpretation. For example, Myant and his collaborators (119) have shown that after the administration of a tracer dose of radioactive iodine its concentration in saliva or in gastric juice depends upon the concentration in plasma. However there is a delay of approximately thirty minutes between the attainment of peak concentration in the plasma and peak concentration in the secretions of the alimentary tract. The authors point out that the thirty-minute lag may account in part for the seeming expansion of the iodide compartment which they have noted in human subjects. Fortunately, this delay in the attainment of equilibrium is not likely to interfere seriously with the interpretation of observations which extend over a period of more than a few hours.

2. *Metabolic Pathways:* The assignment of all of the iodine of the body to three compartments automatically eliminates from consideration certain of the metabolic pathways illustrated in Figure 1. Still further simplification is warranted. While significant quantities of iodide may be lost in the *sweat* during profuse perspiration (178), this avenue of excretion is usually negligible and will not be considered in the discussion which follows. Moreover iodide is so efficiently absorbed by the intestine that only minute traces of iodide ion escape from the body in the *feces* (80, 124). Salter has placed considerable emphasis on the so-called "pulmonary clearance" of iodide, and apparently believes that

very considerable quantities of iodide can escape with water vapor from the lungs and be lost to the body in the *exhaled air* (169). He cites one study in which, 48 hours after the administration of a large therapeutic dose of radioactive iodine to a patient with thyroid cancer, slightly less than 700 microcuries of radioactive iodine remained in the body. At that time the patient was said to exhale almost 2 microcuries per breath. Allowing 20 breaths per minute, this would indicate that iodide was being excreted in the expired air at the patently preposterous rate of about 5 per cent of the amount remaining in the body per minute. In the same paper he later more conservatively estimates that nearly 0.3 per cent of the body's reserve of iodide may be exhaled per hour. The reviewer cannot believe that conditions favorable to the volatilization of iodide are at all likely to occur in the pulmonary epithelium and he is inclined to ascribe Salter's findings to technical error. This belief is strengthened by the fact that Keating was able to find only minute traces of radioactivity in the expired air of patients who had received therapeutic doses of radioactive iodine. The amount recovered was of the order of 0.1 per cent of the dose per 24 hours, and Keating thinks that even this small quantity may have been due to inadvertent contamination with droplets of saliva (79). In the following discussion no further mention will be made of the pulmonary excretion of iodide.

For purposes of mathematical treatment one further simplification will be introduced. The organic iodine excreted in feces and urine will usually be considered as a single entity. The only justification for so doing is that normally the quantity of organic iodine lost by either of these routes is probably small and only a few studies of the urinary or fecal excretion of organic iodine in man are available.

3. *Assumptions Concerning the Distribution of a Tracer Dose of Radioactive Iodine:* Radioactive iodine is commonly administered by mouth to subjects in the post-absorptive state. When so given, absorption is quite rapid (182). Hamilton (69) found that absorption was 80 per cent complete within an hour. Keating and Albert (80) state that the rate of absorption is of the order of 5 per cent per minute. At this rate, absorption would be 90 per cent complete in 45 minutes and 99 per cent complete in 90 minutes. These figures indicate that when radioactive iodine is taken by mouth on an empty stomach there will be a brief but appreciable delay before absorption is complete. When given by vein there is of course no delay in absorption, but time will still be required for the radioactive iodine to become evenly distributed throughout the iodide compartment. Brownell (18) estimates that 50 per cent of the final equilibrium concentration is attained in 15 minutes. At this rate 90 per cent of equilibrium would be reached in 50 minutes or 99 per cent in 100 minutes. In the discussion which follows, both the delay in absorption and the delay in distribution will be disregarded, and for the sake of simplicity it will be assumed that the radioactive iodine is instantaneously and evenly distributed throughout the iodide compartment. This assumption is obviously not legitimate if much importance is to be attached to the fate of radioactive iodine during the first hour or two after administration.

Similar assumptions of rapid and uniform distribution throughout the other

two compartments will be made. In the thyroid gland the validity of this assumption is somewhat dubious. It is well recognized that in the rat the thyroid follicles lying at the periphery of the gland are less active than the central follicles. Although no such anatomical separation of active and inactive follicles has been described in man, it is quite possible that there may be significant variations in the degree of activity from follicle to follicle. Furthermore, although iodide is swiftly oxidized and combined with tyrosine, conversion to thyroxine requires an appreciable period of time even in the rat where the turnover of organic iodine in the thyroid gland is much more rapid than in man (24). Finally, it might seem reasonable to suppose that newly manufactured hormone would be more available for immediate secretion than hormone previously synthesized and stored as colloid within the lumen of the follicles. Despite these theoretical objections, several studies of the rate of loss of radioactivity from the thyroid gland after the administration of a tracer dose have suggested that for periods of at least several weeks a reasonably constant proportion of the amount remaining in the thyroid gland is lost per unit time (21, 83, 179). This constant rate of loss suggests that the labelled organic iodine is fairly evenly distributed throughout the gland.

The rate of distribution of the thyroid hormone from plasma to tissues is very much slower than the rate of distribution of the iodide ion. However, the bulk of unlabelled hormone already in the tissues is so large compared with the quantity of labelled hormone secreted per day that usually the delay in distribution of radioactive iodine throughout the compartment of extrathyroidal organic iodine can be neglected.

The final proof of the legitimacy of these assumptions and simplifications rests squarely on the ability of the simplified model to explain the observed behavior of iodine in the body and to permit deductions which can later be substantiated experimentally. With further improvements in the techniques of measurement it is quite probable that some of these simplifications will have to be abandoned.

The simplified model of iodine metabolism is depicted diagrammatically in Figure 2. In this and several subsequent figures, the size of each cube representing a compartment is proportional to the quantity of iodine contained by that compartment. The width of each arrow representing a pathway of iodine metabolism is proportional to the quantity of iodine which traverses that pathway per unit time. The model is very similar to the one proposed by Brownell (18), to whom the author owes several of the ideas developed in this paper. It differs from Brownell's model in that the compartment for extrathyroidal organic iodine includes the organic iodine of both plasma and tissues, whereas in Brownell's model the organic iodine in tissues was neglected. It differs substantially from the model proposed by Oddie (126). The inclusion by Oddie of a separate compartment for inorganic iodide within the thyroid gland has already been mentioned. Furthermore, he entirely disregarded the organic iodine present in both plasma and tissue. Since Oddie was chiefly interested in the fate of tracer doses of radioactive iodine during the first 24 to 48 hours after administration, his omission of a compartment for extrathyroidal hormone was perhaps justifiable, at least for euthyroid subjects in whom the rate of turnover of iodine in

the thyroid gland is small. Oddie's model will not suffice, however, when the thyroid gland is more active, nor for the interpretation of events occurring over a long period of time.

IV. EQUILIBRIUM STATES AND IODINE BALANCE

Among endocrine organs the thyroid gland is unique in being not only a factory, but also a commodious storehouse for its hormone. This enables it to maintain a steady rate of secretion day after day, despite exceedingly wide fluctua-

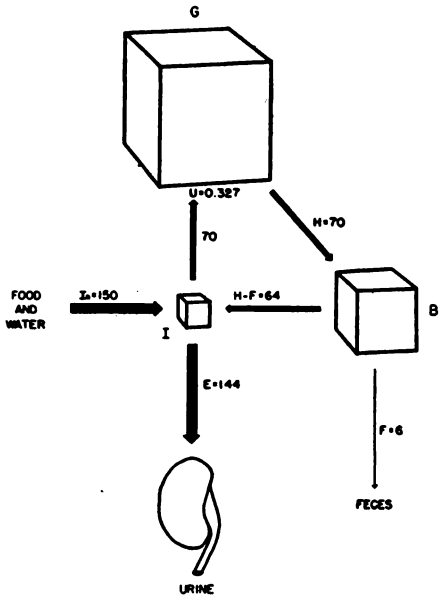

FIG. 2. The simplified model of iodine metabolism upon which is based the discussion in the text. This figure illustrates the metabolism of iodine in a euthyroid subject living in a region where the iodine intake is adequate (Table 3, column 3). The volume of each cube is proportional to the quantity of iodine within the compartment it represents. The width of each arrow is proportional to the micrograms of iodide traversing the designated pathway per day. These rates of transfer are also given by numbers near the arrows. The uptake is given just below the cube representing organic iodine in the thyroid gland. The same mode of construction has been used for Figures 5, 6, 7 and 9. See Section V for explanation of symbols.

tions in the quantity of iodide entering the body. However fortunate this arrangement may be for homeostasis, it seriously hampers the quantitative investigation of thyroid function in man. In the following sections many of the deductions and conclusions will be based on the assumption that the experimental data are obtained from subjects in a state of iodine equilibrium. In other words, it will be assumed that iodine intake precisely balances the loss of iodine from the body, and that the quantity of iodide collected by the thyroid is precisely equal to the quantity of iodine secreted as hormone. In fact, however, the thyroid gland is never in a steady state of equilibrium. Iodide intake is not evenly distributed throughout the 24 hours of the day but is confined almost exclusively

to meal-times. Because of the rapidity with which the iodide ion is collected by the thyroid gland and excreted by the kidneys, this irregular intake of iodine

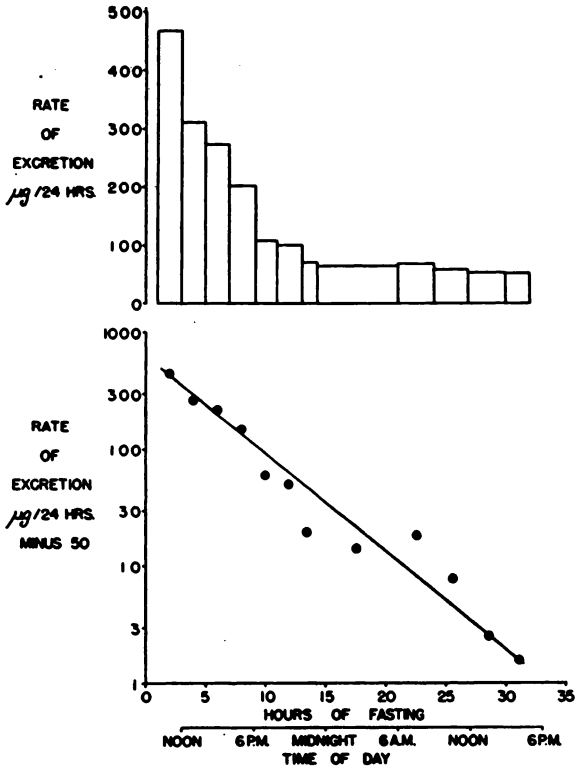

FIG. 3. The renal excretion of iodide during a brief period of starvation. Euthyroid male subject aged 37. The last meal prior to the experiment was a large breakfast, including fish, ending at 9:00 A.M. During the experiment, only distilled water was ingested.

In the upper portion of the figure, the rate of excretion of iodide during successive periods has been plotted as microgm. per day against time. The rate of excretion rapidly approaches a fairly constant value.

In the lower portion of the figure the asymptotic rate of excretion is assumed to be 50 microgm. per day. This value has been subtracted from the observed rates and the differences are plotted on a logarithmic scale against time. The points so obtained can be fitted fairly well by a straight line, thus confirming the assumed value for the asymptote. The 48-hour uptake of radioactive iodine measured at the end of the experiment was 0.401. The mean daily excretion of iodide while the subject was ingesting his usual diet for one day before and six days after the experiment was 148 microgm. H, calculated by equation 15, was 99 microgm./day. F, calculated by equation 28, was 15 microgm./day. In a single experiment of this kind the accuracy with which F can be estimated is low, but it is probably safe to conclude that F is small compared to H.

causes wide fluctuations from hour to hour in the blood concentration of the iodide ion. Corresponding variations occur in the *absolute quantities* of iodide collected by the thyroid gland and excreted by the kidney, even when the *clearances* remain constant (see Figure 3). Consequently, estimates of the total quantity of iodine handled daily by the body must be based upon analyses of

24-hour urine specimens which have been accurately collected. Furthermore, since considerable variations in the iodine content of the diet occur from day to day, it is best to analyze 24-hour urine specimens collected over a period of a week or more. Even this does not insure valid measurements. So great is the quantity of hormone stored in the thyroid gland in comparison with the normal daily secretion of hormone that weeks, or perhaps even months, of iodine deprivation or slight excess may not elicit corresponding changes in thyroid activity. The assumption that iodine equilibrium prevails in any given individual is evidently a dangerous one. Some comfort may be derived from the statistical fact that a subject selected at random is as likely to be in positive as in negative iodine balance, and that as the number of individuals studied increases, the positive and negative errors tend to cancel each other. This statement obviously applies only when the individuals chosen for study represent an unbiased sample of a population in equilibrium with its environment.

V. SYMBOLS, DIMENSIONS, UNITS AND DEFINITIONS

Designation of compartments.

I. Compartment of inorganic iodide, including iodide present in the thyroid gland and in the secretions of the alimentary tract.
G. Compartment of organic iodine in the thyroid gland.
B. Compartment of organic iodine in blood and other extrathyroidal tissues including any thyroxine undergoing enterohepatic circulation.

SYMBOL	DIMENSIONS	UNITS	DEFINITION
Q_I	m	microgm.	Total quantity of iodide in I
Q_G			Total quantity of iodide in G
Q_B			Total quantity of iodide in B
M_G		gm.	Mass of active thyroid tissue
In	m, t^{-1}	microgm./day	Daily intake of iodide
H			Rate of secretion of hormonal iodine
E			Rate of excretion of iodide by the kidney
F			Rate of excretion of organic iodine (chiefly fecal)
(I)	m, l^{-3}	microgm./l.	Concentration of iodide in I
(B)			Concentration of organic iodine in B
V_I	l^{-3}	liters	Volume of distribution of inorganic iodide in I referred to its concentration in plasma
V_B			Volume of distribution of organic iodine in B referred to its concentration in plasma

SYMBOL	DIMENSIONS	UNITS	DEFINITION
t	t	hours	Time
C_K	l^3, t^{-1}	ml./min.	Renal plasma clearance of iodide
C_G			Thyroid plasma clearance of iodide
ϕ	l^3, m^{-1}, t^{-1}	ml./gm.-min.	Blood flow through the thyroid gland
c		l./microgm.-hr.	Liters of V_B cleared of hormone by breakdown and excretion per microgm. of Q_B per hour
K_{IE}	t^{-1}	proportion per hour	Rate constant of renal excretion of iodide.
K_{IG}			Rate constant of iodide uptake by the thyroid gland.
K_I			Rate constant of loss of iodide from I.
K_{GB}			Rate constant of secretion of organic iodide from thyroid.
K_G			Rate constant of net loss of radioactive iodine from thyroid sufficiently long after a tracer dose so that the ratio of specific activity in G to specific activity in B is constant. Rate of biological decay in G.
K_{BI}			Rate constant of breakdown of hormone in B to inorganic iodide.
K_{BF}			Rate constant of excretion of organic iodine.
K_B			Rate constant of loss of organic iodine from B. Rate of biological decay in B.
U	non-dimensional	proportion	Uptake. Proportion of Q_I accumulated by the thyroid.
P			Extraction efficiency of the thyroid gland. Proportion of iodide in ϕ which is used for synthesis of hormone.
C.R.			Conversion ratio. Proportion of total radioactive iodine in plasma which is organic.

It should be noted that each of the rate constants, K, defined above represents the *proportion* of the total quantity of iodine present in a given compartment which leaves that compartment by a given pathway in unit time. The route and direction of transfer are indicated by an appropriate subscript. Thus K_{GB} is the rate constant of transfer *from* G *to* B. From any such rate constant the *time* required for half of the iodine originally present in the compartment

to disappear by the specified route, (the biological half-life), may be calculated by dividing 0.693 (the natural logarithm of 2) by the rate constant.

Symbols used specifically for radioactive iodine will be designated by an asterisk. While quite analogous to the symbols defined above, it must be emphasized that the quantity of radioactive iodine is expressed as a proportion of the administered dose and hence is non-dimensional. In order to avoid difficulty with dimensions, therefore, the symbols employed for radioactive iodine are listed below:

SYMBOL	DIMENSIONS	UNITS	DEFINITION
Q_I^* etc.	non-dimensional	proportion	Proportion of the dose in I etc.
$Q_{\bar{I}}^*$			Proportion of the dose which has been excreted as iodide.
$Q_{\bar{T}}^*$			Proportion of the dose which has been excreted as organic iodine.
H*, E*, F*	t^{-1}	proportion per day	Proportion of the dose secreted as hormone per day etc.
(I)*, (B)*	l^{-3}	proportion per liter	Concentration of radioactive iodine in I etc.

Subscripts are sometimes used to modify the symbols defined above. The meanings of these subscripts are listed below:

0 at time zero.
t at time t.
A in arterial blood.
TV in thyroid vein blood.
s during starvation.
bl during a block of the synthesis of thyroid hormone.

The reviewer is quite aware of the confusion which may arise from using different units for the same dimension. However, it would undoubtedly be even more confusing to express the intake of iodide in gm. per hr. or the renal clearance of iodide in liters per hr. For the most part, therefore, it has seemed wise to express quantities in the units which are traditional and familiar, and to employ unit-conversion factors where necessary in the equations. However, the concentrations of iodine have been expressed as microgm. per liter rather than microgm. per cent, and all non-dimensional quantities have been expressed as proportion rather than as per cent. Since these departures from custom are in the decimal system, they should cause no difficulty.

VI. FACTORS WHICH INFLUENCE THE ACTIVITY OF THE THYROID GLAND

The activity of the normal thyroid gland is regulated chiefly, if not wholly, by the thyrotropic hormone of the anterior pituitary (58, 117, 129, 138). If thyroid activity becomes insufficient to maintain a normal concentration of thy-

roid hormone in the blood stream, the anterior pituitary, by increasing its secretion of thyrotropic hormone, stimulates the thyroid gland to greater activity. Conversely, if the thyroid becomes too active, the increased concentration of circulating thyroid hormone causes inhibition of the secretion of thyrotropic hormone with a compensatory decrease in thyroid function.

The most obvious effect of thyrotropic hormone on the thyroid gland is to cause hypertrophy and hyperplasia which increase the mass of active tissue, M_G. Concomitantly the blood supply also increases. The increase in vascularity may be out of proportion to the increase in mass of active tissue so that the blood flow per gram of tissue, ϕ, increases. Finally the efficiency, P, with which the gland is able to extract iodide from the blood stream and convert it to organic iodine (184) may also be increased. Therefore any factor, alteration of which demands a change in M_G, ϕ or P for maintenance of equilibrium, is a factor which influences thyroid activity. These factors may be expressed in a single equation derived from two separate equations for the rate at which the thyroid clears iodide from the plasma.

The clearance of iodide from *whole blood* by the thyroid gland is equal to the product of the total thyroid blood flow per minute, $M_G\phi$, and the efficiency of extraction P. To convert whole blood clearance, $M_G\ \phi\ P$, to plasma clearance, C_G, it is necessary to correct for the iodide in erythrocytes, which presumably is also available to the thyroid gland (see Section III, A). Taking the water content of plasma as 0.92 and of red blood cells as 0.60, and assuming a normal hematocrit of 0.45 (15), the concentration of iodide in plasma will be about 1.19 times the concentration in whole blood. Hence the *plasma* iodide clearance will be $\frac{1}{1.19}$ times the whole blood iodide clearance:

$$C_G = \frac{M_G \phi P}{1.19} \qquad 1$$

The plasma clearance of iodide by the thyroid will also be equal to the amount of iodide converted to organic iodine per minute divided by the concentration of iodide per ml. of plasma, (I)/1000. But at equilibrium the amount of iodide organically bound by the thyroid per minute will equal the amount secreted as hormone per minute, H/1440. Hence:

$$C_G = \frac{H}{1.44\ (I)} \qquad 2$$

At equilibrium also, the intake of iodine will equal the total excretion of iodine:—

$$In = E + F \qquad 3$$

or

$$E = In - F \qquad 3a$$

Furthermore, the concentration of iodide per liter of plasma may be calcu-

lated from the quantity excreted per minute, E/1440, and the plasma clearance of iodide by the kidney[2], C_K,:

$$C_K = \frac{E}{1.44\ (I)} \qquad 4$$

Substituting the value of E from equation 3a in equation 4, and solving for (I):

$$C_K = \frac{In - F}{1.44\ (I)} \qquad 5$$

$$(I) = \frac{In - F}{1.44 C_K} \qquad 5a$$

Substituting this value for (I) in equation 2:

$$C_G = \frac{C_K H}{In - F} \qquad 6$$

But equations 6 and 1 are both expressions for the plasma clearance of iodide by the thyroid gland, and hence may be equated to each other:

$$\frac{M_G \phi P}{1.19} = \frac{C_K H}{In - F} \qquad 7$$

Equation 7 could be solved for either M_G, ϕ or P. However, the extent to which ϕ and P can increase with increasing thyroid activity is limited. Furthermore P can be profoundly altered by pharmacological agents (see Section X). In contrast, M_G can increase with thyroid activity almost without limit (50) and is probably not directly influenced by drugs other than thyrotropic hormone. It therefore seems logical to solve equation 7 for M_G:

$$M_G = \frac{1.19\ C_K H}{\phi P(In - F)} \qquad 8$$

Equation 8 states that at equilibrium the *mass of active thyroid tissue* is directly proportional to the *renal clearance* of iodide and the *rate of secretion* of the thyroid hormone, and inversely proportional to the *blood flow per gram of tissue*, the *efficiency of extraction* and the difference between *iodide intake* and *excretion of organic iodine*. Together, these seven factors completely determine the activity of the thyroid gland. In subsequent sections each of these factors will be considered in turn.

Equation 8 should not be interpreted to mean that a change in one of the other factors *necessarily* causes a change in the mass of active thyroid tissue. For example, equilibrium could be maintained in the face of an increased intake

[2] Since iodide appears to be excreted by glomerular filtration, and reabsorption (see Section VI, A), the iodide in erythrocytes is not immediately available for excretion during a single passage of blood through the kidney, and no correction of the renal plasma clearance for iodide in erythrocytes need be made.

of iodide by appropriate decreases in blood flow or extraction efficiency, or both, without any change in M_G.

A. *The Renal Clearance of Iodide:* The rate at which iodide is cleared from the plasma by the kidneys may be calculated in the usual manner as the quantity of iodide excreted per minute divided by the concentration of iodide per ml. of plasma (equation 4). If the clearance is to be calculated from chemical determinations of iodide in urine and plasma, the concentration must be deliberately increased by the administration of iodide, since the concentration of inorganic iodide normally present in the plasma is far too small for accurate chemical analysis. This disadvantage is avoided by the administration of carrier-free radioactive iodine which will not appreciably alter the total quantity of iodide within the body. During the first few hours after the administration of a tracer dose, it is usually safe to assume that all of the radioactivity in the blood stream is inorganic iodide, and simple measurements of the total quantity of radioactivity in the plasma and in the urine will suffice for estimation of the renal clearance. However, it is preferable to remove the organic iodine from samples of plasma or serum by precipitating the protein and to measure the radioactive iodide which remains in the supernatant fluid. This precaution becomes essential when the turnover of iodine in the thyroid gland is unusually rapid.

Smith has discussed the theoretical objections to calculation of clearances after the administration of a single dose (176). He believes that reliable results cannot be obtained even when due allowance is made for the logarithmic decrease in blood concentration during the collection periods, and suggests that accurate estimations of renal clearance can be obtained only when the blood concentration is maintained at a constant level by continuous intravenous infusion. Unfortunately the reviewer is unaware of any studies in man during which the blood concentration of either I^{127} or I^{131} was maintained by continuous intravenous infusion. Despite Smith's cogent objections, estimates of the renal clearance of iodide using the single dose technique are probably accurate enough for most purposes.

When serial measurements of radioactive iodine in samples of plasma and urine are available, Berkson and his collaborators (13) consider it more accurate to calculate the renal clearance from the rate of disappearance of radioactivity from the plasma, $K_{IE} + K_{IG}$, the plasma concentration at zero time, $(I)_0^*$, and the proportion of the tracer dose excreted in the urine $1 - U$. The reader is referred to the original paper for a full discussion of the assumptions involved, and for a detailed description of the mode of calculation. In brief, the equation for this method may be derived as follows: By definition, the renal clearance is the proportion of the volume of distribution of iodide which is cleared per minute, multiplied by the volume of distribution. Hence:

$$C_K = \frac{K_{IE}}{60}(1000\ V_I) = \frac{100\ K_{IE} V_I}{6} \qquad 9$$

But K_{IE}, the rate constant of excretion, is equal to the proportion of the dose

excreted in the urine, multiplied by the total rate of disappearance from the plasma:

$$K_{IE} = (1 - U)(K_{IE} + K_{IG}) \qquad 10$$

And since at zero time the entire dose is assumed to be evenly distributed

TABLE 1

The renal clearance of radioactive iodide

SUBJECTS	NUMBER OF SUBJECTS	RENAL CLEARANCE OF I^{131} ML. OF PLASMA PER MINUTE			REFERENCE NUMBER
		Minimum	Maximum	Mean*	
Euthyroid	7	11	44	31	123
Euthyroid (Medical Students)	12	21	53	36	119
Euthyroid	9			33.3 ± 3.1	105
Euthyroid weighted mean	28			34	
Euthyroid Renal Function Impaired	12			8.7 ± 1.6	105
Hyperthyroid	5				28
I^{127} carrier					
0.001 mgm.		40	49	44.3 ± 2.0	
0.1 mgm.		26	53	36.9 ± 4.5	
1.0 mgm.		29	52	34.3 ± 5.6	
10.0 mgm.		25	52	37.1 ± 4.9	
Hyperthyroid	16			32.7 ± 1.0	105
Hyperthyroid	11	10	46	27	123
Hyperthyroid weighted mean	32			32	
Myxedema	5			17.9 ± 1.6	105

* When available, the standard error of the mean is also given.

throughout the volume of distribution:

$$V_I = \frac{1}{(I)_0^*} \qquad 11$$

Substituting the values from equations 10 and 11 in equation 9,

$$C_K = \frac{100\,(1 - U)(K_{IE} + K_{IG})}{6(I)_0^*} \qquad 12$$

Berkson and his collaborators have shown that the agreement between these two methods of calculation is usually very satisfactory. In a series of 40 patients the mean clearance determined by the classical method was 31.7 ml. of plasma per minute. The mean clearance by the method just described was 30.7 ml. of plasma per minute.

Table 1 summarizes values for the renal clearance of iodide from the plasma under various circumstances. The mean clearance among euthyroid subjects

is about 34 ml. of plasma per minute with considerable variation from individual to individual. For convenience in calculation, the mean normal value will henceforth be taken as 33⅓ ml. of plasma per minute. It is also evident from Table 1 that no significant change in the renal iodide clearance occurs during hyperthyroidism. However, in myxedema the renal clearance of iodide may be significantly decreased, and in patients with renal disease the ability of the kidneys to clear iodide from the blood stream is often greatly impaired.

The clearance of iodide by the human kidney is very much greater than the clearances of chloride, bromide or thiocyanate, but is still only about one quarter of the glomerular filtration rate. Presumably this means that approximately three quarters of the iodide filtered by the glomeruli is reabsorbed by the renal tubules.

The renal clearance of iodide is remarkably constant over an enormous range of plasma concentrations. When the plasma concentration is raised to 100,000 microgm. per liter by the administration of carrier iodide, the renal clearance of radioactive iodine is approximately the same as when the radioactive iodine is given without carrier (28). Furthermore, no reduction in renal clearance occurs in regions of iodine deficiency where the mean concentration of iodide ion in the plasma may be as low as 0.2 microgm. per liter (179). This complete indifference of the kidney to the need for conservation of iodide imposes a heavy burden upon the thyroid gland when the supply of iodine is restricted.

In man the renal excretion of the iodide ion, unlike that of the bromide ion, is but little influenced by extreme variations in the quantity of chloride being excreted simultaneously (159). In contrast, in the dog the renal clearance of iodide is largely determined by the rate of chloride excretion. The clearance of iodide in this species may rapidly change from less than 0.1 ml. of plasma per minute when chloride intake is restricted, to over 30 ml. of plasma per minute when large quantities of chloride are administered (156).

Because of the keen competition between the thyroid gland and the kidneys for the available supply of iodide, impairment of renal function severe enough to reduce the renal iodide clearance makes it much easier for the thyroid gland to obtain its daily allotment of iodide. Renal impairment should therefore provide partial protection from thyroid enlargement due to iodine deficiency since a decrease in C_K will tend to counterbalance a decrease of In (see equation 8).

B. *The Rate of Secretion of the Thyroid Hormone:* The rate of secretion of the thyroid hormone cannot be directly measured. Even if it were technically possible to obtain pure samples of venous blood from the thyroid gland in man, the arterio-venous difference in concentration of thyroid hormone would be far too small to be detectable by present analytical methods. If the plasma entering the thyroid gland contained 50 microgm. of protein-bound iodine per liter and if the rate of secretion of thyroid hormone were normal, the plasma leaving the gland would contain about 51 microgm. per liter, an increase of only 2 per cent. Reliance must therefore be placed on indirect methods for estimating the rate of hormone secretion.

1. *Methods Based on Substitution Therapy:* In the past it has been customary

to consider the quantity of exogenous hormone which must be supplied daily to a patient with complete myxedema as equal to the normal quantity of hormone secreted daily by the thyroid gland. This involves the assumption that the exogenous hormone is utilized as efficiently as endogenous hormone. Plummer and Boothby (136) estimated that approximately 300 microgm. of racemic thyroxine are required daily to maintain patients with severe myxedema in a euthyroid state. In a very thorough study of two patients with myxedema Thompson and his collaborators found that the daily requirement was about 325 microgm. of racemic thyroxine (191). Since d-thyroxine is only one tenth as potent as l-thyroxine (134), 325 microgm. of the racemic drug is equivalent to 179 microgm. of the naturally-occurring levorotatory isomer, or to 116 micrograms of hormonal iodine per day.

Somewhat smaller amounts of hormone appear to suffice when desiccated thyroid is administered by mouth. From his very wide experience, Means estimates the daily requirement as 100 mgm. of U.S.P. thyroid (107). Winkler and his collaborators (201) found that an increase of serum iodine averaging 2.0 microgm. per cent occurred for each 65 mgm. of thyroid administered to patients with hypothyroidism. It would therefore require approximately 160 mgm. of thyroid daily to increase the concentration of iodine in the serum from zero to a normal level of 5 microgm. per cent. Among 25 normal subjects studied by Greer (59), the daily amount of thyroid needed to decrease the uptake of radioactive iodine to less than 10 per cent of the administered dose was 65 mgm. in 9 subjects, 130 mgm. in 12 subjects, 195 mgm. in 2 subjects, and 260 mgm. in 2 subjects. The mean quantity required for marked (but not necessarily complete) suppression of thyroid activity was therefore approximately 120 mgm. per day. As a compromise between these three estimates, 130 mgm. may be taken as the mean quantity of thyroid needed per day for complete substitution therapy in patients without functional thyroid tissue. Although the total quantity of organic iodine in 130 mgm. of U.S.P. thyroid is 260 $\pm$ 39 microgm., only about one third of this, or 87 microgm., is present as thyroxine. The remainder is present as iodinated tyrosine, which is physiologically inert.

The supposed discrepancy between the quantity of thyroxine required when given as the pure substance by vein and when given by mouth in the form of desiccated thyroid has long been a matter of comment. The statement is often made that the potency of dried thyroid is greater than can be accounted for by its thyroxine content, the implication being that in some mysterious way the other iodine-containing compounds become endowed with calorigenic activity (93, 111). But when due allowance is made for the relative activity of the optical isomers of thyroxine, the discrepancy between the potency of thyroid by mouth and thyroxine by vein almost disappears. Furthermore, recent investigations of the fate of thyroxine in animals and in humans provide a reasonable explanation for whatever small discrepancy remains. Gross and Leblond (62) have shown that in the rat over two thirds of an unphysiologically large dose of radioactive thyroxine is excreted in the feces during 24 hours. When physiological doses are used, much smaller amounts are excreted. In man, thyroxine is usually given by vein and, since the rate at which it leaves the blood stream is rather slow

(2, 122, 153), abnormally high concentrations which may favor excretion by the liver are undoubtedly present in the plasma for several hours after an intravenous injection.

An ingenious experiment by Myant and Pochin (122) casts further doubt on the equivalence of injected thyroxine and endogenous thyroid hormone. In three euthyroid subjects the behavior of synthetic dl-thyroxine labelled with radioactive iodine was compared with radioactive hormone synthesized by the thyroid glands of three thyrotoxic patients who had received large therapeutic doses of radioactive iodine. Three days after administration of the therapeutic doses 20 ml. aliquots of plasma from the thyrotoxic patients were injected into the normal subjects. The "natural thyroxine" disappeared from the blood stream at a much slower rate than synthetic radioactive thyroxine. Twenty-four hours after injection, the mean concentration of natural thyroxine was 14.8 per cent of the dose per liter of plasma, while the mean concentration of synthetic thyroxine was only 3.6 per cent of the dose per liter of plasma. Since the dose of synthetic thyroxine was only 80 microgm., the blood concentration, even immediately after injection, was probably not high enough to favor increased excretion by the liver. The observed difference in rate of disappearance cannot be ascribed to the use of racemic thyroxine, since in two control experiments the distribution of pure synthetic l-thyroxine was similar to the distribution of the racemic compound. By demonstrating that the majority of the organically bound radioactivity could be extracted with butanol in which thyroglobulin is not soluble, the authors also excluded the possibility that the samples of serum from the thyrotoxic patients might have contained thyroglobulin rather than thyroxine. They concluded that thyroglobulin could not have constituted more than a small fraction of the radioactive iodine in the plasma. The authors suggest that the linkage between injected thyroxine and the plasma proteins may differ from the linkage which occurs when thyroxine is secreted from the thyroid gland into the blood stream. This explanation is also supported by the experiments of Salter and Johnson (168). However, Gordon and his collaborators (53) have recently demonstrated that when serum from patients treated with large doses of radioactive iodine is subjected to electrophoresis on filter paper, practically all of the organic iodine accompanies α_1 globulin. Thyroxine mixed with plasma *in vitro* behaved in precisely the same fashion. This constitutes strong presumptive evidence that the binding of exogenous thyroxine is similar to the binding of endogenous hormone.

While the evidence is conflicting, it is likely that injected thyroxine is not utilized quite as efficiently as hormone released from the thyroid gland. When desiccated thyroid is given by mouth, the absorption of hormone from the gastro-intestinal tract may be slow enough to permit more efficient utilization. It seems highly probable that the calorigenic activity of desiccated thyroid is fully accounted for by its thyroxine content. However, it is unlikely that absorption and utilization is 100 per cent efficient. Consequently, the 85 to 90 microgm. of l-thyroxine iodine required daily in this form is probably somewhat greater than the daily secretion of hormonal iodine from the normal thyroid gland.

2. *Methods Based on the Uptake of Radioactive Iodine by the Gland and the*

Excretion of Stable Iodine in the Urine: Since the thyroid gland does not distinguish between I^{131} and I^{127}, the proportion of a tracer dose of radioactive iodine which is accumulated by the thyroid is also the proportion of *all* of the iodide in the body which is accumulated. If equilibrium is maintained, it is likewise the proportion of all of the iodide *entering* the iodide compartment which is accumulated. The iodide entering is the sum of In, the iodide absorbed from the gastro-intestinal tract, and H − F, the iodide returned to the iodide compartment by breakdown of thyroid hormone in the tissues. The total quantity of iodide entering the thyroid gland in 24 hours is therefore U(In + H − F). But at equilibrium the quantity of iodide entering the gland is equal to the quantity secreted as hormone. Hence:

$$H = U(In + H - F) \qquad 13$$

From equation 3:

$$In = E + F \qquad 3$$

substituting this value in equation 13:

$$H = U(E + H) \qquad 14$$

Solving for H:

$$H = \frac{UE}{1 - U} \qquad 15$$

The same equation can be derived more simply, but perhaps less instructively, from the fact that the ratio of iodide collected to iodide excreted is $\frac{U}{1 - U}$. Hence, at equilibrium:

$$\frac{H}{E} = \frac{U}{1 - U} \qquad 16$$

The dangers inherent in the assumption of equilibrium have already been discussed. Consequently, equation 15 is most useful when H can be calculated from studies of a considerable number of euthyroid individuals. For this purpose a logarithmic transformation is particularly useful. Solving equation 15 for E,

$$E = \frac{H(1 - U)}{U} = H\left(\frac{1}{U} - 1\right) \qquad 17$$

$$\log E = \log H + \log\left(\frac{1}{U} - 1\right) \qquad 18$$

It is evident from equation 18 that a plot of log E against $\log\left(\frac{1}{U} - 1\right)$ should give a straight line with a slope of unity and that the intercept of this line with the zero axis for $\log\left(\frac{1}{U} - 1\right)$ should provide an estimate of log H. In practice

the uptake of radioactive iodine and the excretion of I^{127} in 24-hour urine specimens are measured in a number of euthyroid individuals, and the observed values for E and U are appropriately transformed and plotted according to equation 18. The best straight line with a slope of unity is fitted to the points and log H is read as the value of log E when $\log\left(\frac{1}{U} - 1\right)$ is zero (*i.e.*, when U = 0.5).

This mode of calculation has been used in the analysis of data obtained from euthyroid subjects in a region of iodine deficiency (179). A crude estimate of 45 microgm. per day was obtained for the normal rate of secretion of hormonal iodine. The data are subject to a number of corrections which will undoubtedly increase the calculated value. Moreover, because of the difficulty of persuading a large number of normal subjects to collect accurate and complete 24-hour urine specimens, this method is almost certain to underestimate the true value for the rate of secretion of the thyroid hormone.

If the rate of excretion of organic iodine is known, or if it is small enough to be negligible, the rate of secretion of hormone from the thyroid gland may also be calculated from the excretion of I^{127} in fasting subjects. This method will be discussed below in the section on the excretion of organic iodine.

3. *Methods Based on Specific Activity:* Stanley has pointed out that the quantity of I^{127} accumulated by the thyroid gland can be calculated from the excretion of I^{127} in the urine and the specific activity of the urine (181). Since the thyroid and the kidneys do not distinguish between stable and radioactive iodine, at any given moment the iodide being accumulated by the thyroid gland or being excreted in the urine must have the same specific activity as the iodide in the blood stream. Hence:

$$\frac{I^{131}\text{ entering thyroid}_t}{I^{127}\text{ entering thyroid}_t} = \frac{I^{131}\text{ in urine}_t}{I^{127}\text{ in urine}_t} = \frac{(I)^*_t}{(I)_t} \qquad 19$$

and,

$$I^{127}\text{ entering thyroid}_t = \frac{(I^{127}\text{ in urine})_t\,(I^{131}\text{ entering thyroid})_t}{(I^{131}\text{ in urine})_t} \qquad 20$$

After a single dose of radioactive iodine it is obvious that the blood concentration of I^{131} will fall rapidly as the isotope is trapped by the thyroid gland or excreted in the urine. The concentration of I^{127}, on the other hand, will be influenced not only by the activity of the thyroid and the kidneys but also by the quantity of I^{127} being added to the iodide compartment continually by breakdown of hormone in the tissues, and intermittently by absorption from the gastro-intestinal tract. Consequently, the specific activity will decrease rather rapidly with the passage of time. This will not affect the validity of equations 19 and 20, since the change in specific activity will be the same for each of the three components of the equation. Since equation 20 is valid for each instant of time, it is also valid for the total amount collected and excreted during any given *interval*

of time, provided that C_K and C_G remain constant. If enough time is allowed for all of the radioactive iodine to be excreted or accumulated, the method of equation 20 reduces to that of equation 15. However, equation 20 is somewhat more versatile than equation 15. Since it does not require the assumption of equilibrium, it can be used for the calculation of the quantity of stable iodide entering the thyroid gland during brief periods of time. It thus facilitates the investigation of factors which alter the quantity of iodide accumulated. Using this method, Stanley has been able to demonstrate the transient influence of iodide in food or medication on the quantity of iodide entering the thyroid. It should be evident from the previous discussion, however, that reliable estimates of the rate of *secretion* of hormone cannot be obtained when equation 20 is applied to observations made during a short period of time. For example, Stanley reported that the rate of accumulation of stable iodide in 14 euthyroid subjects who had not received additional iodide varied from 3 to 19 microgm. per hour, averaging 10 microgm. per hour, for periods of from 6 to 28 hours. If the mean rate of 10 microgm. per hour were assumed to be representative of iodide accumulation over a long period of time, it would imply that the normal rate of secretion was approximately 240 microgm. of hormonal iodine per day. This estimate is considerably higher than the ones discussed previously and probably indicates that during the period of study most of Stanley's subjects were accumulating more than their equilibrium allotment of iodide.

Theoretically it should be possible to calculate the rate of secretion of hormonal iodine from the thyroid gland by measuring the increase in the excretion of stable iodide which occurs when the synthesis of hormone is blocked by the administration of a drug such as 1-methyl-2-mercaptoimidazol. For this method of calculation, it is necessary to assume that the sole action of the drug is to inhibit the uptake of iodide by the thyroid gland, and that loss of iodine from the gland and return of iodide from the tissues remain constant throughout the period of study. It is also necessary to determine the uptake of radioactive iodine by the blocked gland as a measure of the degree of block produced. During the block the quantity of iodide excreted per day in the urine will be equal to the proportion of the tracer dose excreted multiplied by the quantity of iodide entering the iodide compartment:

$$E_{bl} = (1 - U_{bl})\,(In + H - F) \qquad 21$$

Substituting in equation 21 the value for $In - F$ from equation 3a, and solving for H:

$$E_{bl} = (1 - U_{bl})(E + H)$$

$$H = \frac{E_{bl}}{1 - U_{bl}} - E \qquad 22$$

If the block is complete, U_{bl} becomes equal to 0, and the equation reduces to:

$$H = E_{bl} - E \qquad 23$$

Unexpected difficulties were encountered when this mode of calculation was

applied (179). The difference between the excretion of stable iodide before and during the administration of l-methyl-2-mercaptoimidazol was much larger than the daily secretion of hormone estimated by previous methods. Furthermore, it is possible to predict from U_{bl} and U the ratio to be expected between E_{bl} and E. Substituting in equation 22 the value for H from equation 15

$$\frac{UE}{1-U} = \frac{E_{bl}}{1-U_{bl}} - E \qquad 24$$

Solving equation 24 for $\frac{E_{bl}}{E}$:

$$E\left(\frac{U}{1-U}+1\right) = \frac{E}{1-U} = \frac{E_{bl}}{1-U_{bl}}$$

$$\frac{E_{bl}}{E} = \frac{1-U_{bl}}{1-U} \qquad 25$$

The observed ratio of E_{bl} to E was considerably greater than the ratio predicted by equation 25. This calls into serious question the validity of the basic assumption that the blocking agent did not affect the rate of loss of iodine from the thyroid gland. It seems possible that blocking agents of this type increase the rate at which iodine leaves the gland. Albert and Rawson have suggested that they may potentiate the action of thyrotropic hormone (4). If so, the extra iodine lost from the thyroid would presumably be secreted as thyroxine. This should cause a *gradual* increase in the excretion of iodide. But in the study described above, the increased excretion of iodide occurred rather promptly. It seems more probable that the extra iodine leaves the gland either as iodinated tyrosine or as iodide derived from the breakdown of iodinated tyrosine. This peculiar phenomenon deserves further investigation.

4. *Other Methods:* Salter presents several equations for the calculation of what he terms the "thyroxine metabolic turnover rate" or "TMR" which he defines as the daily production of l-thyroxine expressed as micrograms of organic iodine (164, 166, 167). Unfortunately, he gives no satisfactory derivation for these equations and does not even define some of the symbols employed. Furthermore, it is easy to show by dimensional analysis that two of his equations cannot possibly be equivalent mathematically.[3] It would therefore seem pointless to discuss them further.

[3] Salter states (167):—"Theoretically,

$$\text{TMR} = \frac{\text{Plasma volume}}{100} \times \text{SHI ln SHI} \times K = K \frac{\text{serum radioactivity}}{\text{per 100 ml.}} \times \left(\frac{\text{plasma volume}}{100}\right)^2$$

Where
TMR = daily production of l-thyroxine (expressed as micrograms of organic iodine);
SHI = serum protein-bound iodine in micrograms per 100 ml.; and
K = the labelling constant which is close to 0.4 in value."

If we assume that by $\frac{\text{"serum radioactivity}}{\text{per 100 ml."}}$ is simply meant "serum radioactivity per 100 ml." the second and third portions of this "equation" may be reduced to:—

While it is not possible at the present time to give a final value for the normal rate of secretion of the thyroid hormone in man, we are fortunate in having one estimate (based upon replacement therapy) which is probably too high, and another estimate (based upon the excretion of stable iodine in the urine and the uptake of radioactive iodine) which is certainly too low. The true value must lie somewhere between these two independent estimates. Pending more precise measurements, we may accept a value of 70 microgm. of thyroxine iodine per day as the mean rate of secretion in the normal human. This figure is certainly of the correct order of magnitude and is probably rather close to the true value.

C. *The Daily Intake of Iodine:* There is no such thing as a "normal" intake of iodine. The ability of the thyroid gland to compensate for changes in iodine supply is so great that it can maintain a normal rate of secretion whether the intake be 10 microgm. per day, as in regions of severe iodine deficiency, or several million microgm. per day, as when large doses of iodide are administered in the treatment of syphilis and fungus infections. The reviewer will adopt the chauvinistic attitude that the iodine intake in Boston and similar seacoast regions is normal. Even in such regions of natural iodine abundance, great variation must be anticipated because of dietary vagaries, the deliberate or unwitting use of iodized salt, the employment of iodate in certain bread conditioners, and the countless ointments, toothpastes and drugs which contain relatively enormous quantities of iodine.

In persons eating a normal mixed diet it is practically impossible to obtain reliable measurements of iodine intake by the chemical analysis of food and water. The formidable difficulties of sampling foodstuffs without bias, and the decreased reliability of analytical results when large amounts of fat are present in the material to be analyzed, have discouraged recent investigators from attempting the direct measurement of iodine intake. In the next section it will be shown that the loss of organic iodine from the body in both urine and feces is probably small. Reasonably reliable estimates of intake may therefore be obtained by chemical analysis of 24-hour urine specimens. In Table 2 are summarized the results of several studies of the daily excretion of iodide in regions without endemic goiter. Most of the values were obtained from the excellent monograph by McClendon (101). The mean is close to 150 microgm. per day. The mean intake of iodine in food and water is therefore slightly greater than this. For ease in calculation, 144 microgm. per day will be taken as the "normal" daily excretion of iodide in the urine.

The effects on thyroid function of iodine deficiency (see Section VIII, F) and of iodine excess (see Section X, B) will be discussed later.

D. *The Excretion of Organic Iodine:* In the previous discussion it has been convenient to represent the organic iodine lost from the body as a single entity.

SHI ln SHI = serum radioactivity per 100 ml. $\left(\frac{\text{plasma volume}}{100}\right)$

The dimensional "equation" for this is:—

$m, l^{-3} = l^{-3}, l^3$

which is impossible.

It is now necessary to consider urinary and fecal losses separately since there is evidence that small quantities of organic iodine may be excreted by both of these routes.

1. *Urine:* Before the advent of radioactive iodine, attempts to demonstrate organic compounds of iodine in the urine of normal or hyperthyroid subjects by chemical methods had repeatedly failed, and it was generally conceded that most, if not all, of the urinary iodine was inorganic (40, 43). This comfortable belief has recently been challenged by Rall (142) who has studied the excretion of radioactive iodine in urine samples fractionated by chemical and physical methods. In the urine of normal individuals only minute traces of iodine precipitable with zinc hydroxide were found 72 hours after a tracer dose. At this

TABLE 2

The urinary excretion of iodine in regions free of endemic goiter

INVESTIGATOR	LOCALITY	IODINE IN 24-HOUR SAMPLE	REFERENCE
		microgm.	
Von Fellenberg	Vik i Sogn, Norway	146	McClendon (101) page 53
Lunde	Vik i Sogn, Norway	173	McClendon (101) Table 53
Reith	Barendrecht, Holland	186	McClendon (101) Table 54
Kupzis	Latvia	(81 to 157)	McClendon (101) Table 54
Hercus and Roberts	New Zealand	49	McClendon (101) Table 54
Triebart	Batavia	126	McClendon (101) Table 54
Triebart	Java	135	McClendon (101) Table 54
Bruger	New York, N. Y.	191	Salter (162) page 79
Stanbury *et al.*	Boston	125	Unpublished data
Liek	Danzig	343	Curtis *et al.* (35) Table 2
Scheringer	Berlin	141	Curtis *et al.* (35) Table 2
Moore	New Orleans	117	Curtis *et al.* (35) Table 2
Von Fellenberg	Forte dei Marmi, Italy	72	Curtis *et al.* (35) Table 2
Mean (omitting value in parenthesis)		150.3	

time practically all of the radioactive iodide originally administered had presumably either been excreted by the kidneys or accumulated by the thyroid gland. It seems probable, therefore, that the radioactivity appearing at 72 hours was derived chiefly from labelled thyroid hormone which had been synthesized and secreted by the thyroid gland and metabolized by the tissues. This suggests that in normal subjects the urinary excretion of organic iodine is negligible. In hyperthyroid subjects, however, approximately 25 per cent of the radioactive iodine in the urine 72 hours after a tracer dose was precipitable with zinc. Since in hyperthyroidism the rate of accumulation of iodide in the thyroid gland is so rapid, it is almost certain that the radioactivity in the urine represented only iodine derived from labelled hormone. Indeed, Rall (142) points out that the appearance of precipitable radioactive iodine in the urine closely parallels the appearance of precipitable radioactivity in the serum (104). Since Rall did not report the uptake of radioactive iodine in these subjects, it is difficult to calcu-

late the proportion of the organic iodine secreted by the thyroid gland which was being lost from the body in the urine. However, if it be assumed that the mean uptake was 75 per cent, the following calculation will provide a rough estimate of the proportion of hormone lost in the urine as organic iodine:

Let X equal the total amount of I^{131} in the urine at 72 hours.

Then 0.75X equals the inorganic I^{131} in the urine.

But this represents only 0.25 of the total amount of I^{131} returned to the blood stream by breakdown of labelled hormone in the tissues.

Hence, total amount of I^{131} returned to the iodide compartment $= \frac{0.75\ X}{0.25} =$ 3X.

Organic I^{131} lost in urine $= 0.25$ X.

Total labelled hormone turnover $= 3.25$X.

Proportion lost in urine as organic $I^{131} = \frac{0.25X}{3.25X} = 0.077$.

In this calculation, fecal excretion has been neglected, and it has been assumed that at 72 hours the proportion of the radioactivity in the urine which is organic is the equilibrium proportion. Actually Rall's figures suggest that the proportion may still be increasing slowly at 72 hours. However, insofar as this calculation is valid it suggests that even in hyperthyroidism less than 10 per cent of the hormone secreted by the thyroid appears in the urine as organic iodine.

If the values for thyrotoxicosis listed in Table 3 be taken as representative, the turnover of hormone per day (neglecting fecal loss) would be 597 microgm. According to the calculation above, 46 microgm. of this might be excreted in organic form. In addition, 199 microgm. of inorganic iodide would be excreted. Of the total iodine in the urine $\frac{46}{245}$ or 0.19 would be organic. If as much as 19 per cent of the iodine being excreted in hyperthyroidism is organic iodine, it is rather surprising that it has not been detected by chemical methods. The problem should certainly be re-examined with the improved chemical techniques which are now available.

Rall also separated the organic iodine in the urine into thyroxine-like and diiodotyrosine-like fractions. Unfortunately, the tabulation of results which he presents does not indicate at what interval after the tracer the urines were collected. In one patient with myxedema, three euthyroid subjects, and six patients with hyperthyroidism, only 2 per cent or less of the radioactive iodine in the urine was found in the thyroxine fraction. However, in four other patients with hyperthyroidism from 3 to 10 per cent behaved like thyroxine. By adding carrier thyroxine to butanol extracts of urine and recrystallizing to constant specific activity, and by chromatographic separation on filter paper, Rall appears to have conclusively demonstrated the presence of at least some thyroxine in the urine.

The proportion of the radioactive iodine in the urine which was recovered in the diiodotyrosine fraction was extremely variable, ranging from 1.4 per cent in a patient with myxedema, to as much as 68 per cent in a patient with exoph-

thalmic goiter. In the 10 patients with hyperthyroidism the mean proportion was 28 per cent. It is difficult to reconcile these observations with a later study by Albert and Keating (3) who clearly showed that when radioactive diiodotyrosine was injected intravenously only about 6 per cent of the administered dose was excreted unchanged in the urine. The remainder was rapidly broken down in the tissues and excreted as inorganic iodide. Hence, even if the thyroid hormone were quantitatively converted to diiodotyrosine in the tissues, no more than 6 per cent of the urinary iodide derived from hormone would be excreted as diiodotyrosine.

While the reviewer is prepared to grant the presence of minute amounts of thyroxine in urine, he is inclined to believe that the majority of the diiodotyrosine found by Rall represents some sort of an artifact. This supposition is strengthened by a study of the iodine compounds excreted after the intravenous administration of radioactive dl-thyroxine (2). Of the total radioactive iodine in the urine, about 85 per cent was inorganic iodide. Ten per cent behaved like thyroxine iodine and only 5 per cent or less appeared to behave like diiodotyrosine iodine. In a similar study, Myant and Pochin (122) found that after the intravenous administration of labelled thyroxine, 9 per cent of the radioactive iodine in the urine was thyroxine iodine as judged by butanol extraction. They did not attempt to identify diiodotyrosine but assumed that the remaining 91 per cent was inorganic iodide. In evaluating these reports it must be recalled that exogenous thyroxine may not be metabolized in quite the same manner as the normal thyroid hormone (see Section VI, B, 1).

Finally, the renal excretion of any considerable quantity of thyroxine may be doubted on theoretical grounds. In the blood stream thyroxine is so completely bound to plasma protein that it cannot be removed by ultrafiltration or prolonged dialysis (157, 174, 187). While this by no means precludes the existence of an exceedingly small concentration of unbound thyroxine (see Section XI, A), it probably prevents the kidney from removing more than traces of thyroxine from the blood stream. This argument, however, loses much of its force when it is realized that in a hyperthyroid patient with a renal plasma flow of 700 ml. per minute and a concentration of 14 microgm. of protein-bound iodine per 100 ml. of plasma, 141,000 microgm. of protein-bound iodine would flow through the renal blood vessels in 24 hours. It is obvious that removal of a very minute proportion of this could account for the quantities of thyroxine found by Rall in the urine of patients with hyperthyroidism.

In summary, the excretion of organic iodine in the urine can safely be disregarded in normal subjects. In hyperthyroidism the extent and nature of organic iodine in the urine require further investigation. Even in hyperthyroidism probably no great errors in calculation will occur if all of the urinary iodine is assumed to be inorganic iodide.

2. *Feces:* Early attempts to determine chemically the quantity of iodine normally excreted in the feces have been reviewed by Salter (162). It is doubtful that any of these analyses were sufficiently accurate to be trustworthy.

Since the original report by Krayer (86) many investigators have found that,

after the parenteral administration of thyroxine, a very large proportion of the iodine may be excreted in the feces. Even with doses of radioactive l-thyroxine as small as 0.001 to 0.07 microgm., Gross and Leblond (63) found that rats excreted approximately 30 per cent of the administered radioactive iodine in the feces within 24 hours. Only a fraction of this was organic. The authors estimate that of the thyroxine present in the body of the rat at any one time less than $\frac{1}{5}$ is likely to be lost in the feces as thyroxine itself. With larger unphysiological doses (20–7,000 microgm.) a majority of the administered iodine appeared in the feces within 24 hours, and the proportion which behaved chemically like thyroxine was also much increased. In the rat, the appearance of thyroxine in the feces is due largely, if not entirely, to secretion of thyroxine by the liver into the bile (62, 86). Taurog and his collaborators (185) have recently demonstrated that in both dogs and rats the liver conjugates thyroxine, probably with glucuronic acid. Unless large doses are given, very little of the thyroxine in the bile is free. However, the conjugate is apparently hydrolyzed in the intestine, perhaps by the glucuronidase of the intestinal bacteria, so that only free thyroxine is found in the feces. The conjugate was also identified in the bile of rats given radioactive iodide, thus proving that endogenous thyroxine is also conjugated by the liver.

The possible importance of biliary excretion of thyroxine in man has also been suggested by two experiments in which the distribution of thyroxine after parenteral administration was studied. Albert and Keating (2) injected 1 mgm. of labelled thyroxine into a cretin who had been maintained in a euthyroid state with desiccated thyroid. During the following 8 days 41.3 per cent of the dose was excreted in the urine, the majority of it as iodide, while 11.5 per cent of the dose was excreted in the feces, most of it as precipitable iodine. Of the total excreted, therefore, approximately 22 per cent was fecal. In a similar study, Myant and Pochin (122) injected 80 microgm. of labelled thyroxine intravenously into 3 normal subjects, and recovered approximately 10 per cent of the radioactivity in the feces within a 3-day period. They did not attempt to determine the chemical nature of the fecal iodine. While these studies clearly demonstrate that in man, as in the rat, a very significant proportion of a dose of exogenous thyroxine may be lost from the body in the feces, they do not necessarily indicate that this is an important excretory route for endogenous thyroid hormone.

After the initial collection and excretion of a tracer dose of radioactive iodine has been completed, a comparison of radioactivity in urine and feces should permit calculation of the proportion of the endogenous hormone which is lost by fecal excretion. Unfortunately, the reviewer is not aware of any such studies with tracer amounts of radioactive iodine. However Dr. A. Stone Freedberg (47) has kindly supplied the following unpublished data from a patient with thyrotoxicosis who was given a therapeutic dose of radioactive iodine. Comparisons of urinary and fecal excretion were made on the 5th, 6th, 8th, 9th and 10th days following treatment. The mean urinary excretion was 4.66 per cent of the dose per day, while the mean fecal excretion was 0.67 per cent of the dose per day. Thus fecal loss constituted 12.5 per cent of the total excreted.

Because of the difficulty of measuring the loss of organic iodine in the feces directly, two indirect methods of calculation will now be presented. While the accuracy of these methods is probably not very great, they should at least indicate whether fecal loss of organic iodine is a *major* pathway of iodine metabolism in man.

If the intake of iodide is abruptly and completely stopped, the only remaining source of iodide in the urine will be breakdown of hormone in the tissues. The concentration of iodide in the blood will therefore decline towards an asymptotic value. When the asymptote has been reached, the quantity of iodide appearing in the urine should equal the quantity of iodide being returned to the iodide compartment from the tissues, multiplied by the proportion destined for renal excretion:

$$E_s = (1 - U)(H - F) = H(1 - U) - F(1 - U) \qquad 26$$

but from equation 15:

$$H(1 - U) = EU \qquad 15a$$

Hence:

$$E_s = UE - F(1 - U) \qquad 27$$

Solving for F:

$$F = \frac{UE - E_s}{1 - U} \qquad 28$$

It should therefore be possible to calculate the excretion of organic iodine in the feces from a comparison of the urinary excretion of iodide before and during a fast and from the uptake of radioactive iodine. Because of the rapidity with which the kidneys and thyroid clear iodide from the blood stream, the period during which the intake of iodine is eliminated need not be long. In normal subjects the asymptotic rate of excretion may be estimated from the iodine content of serial samples of urine collected every two or three hours during a 30-hour period of fasting (Figure 3). Much shorter periods should suffice for patients with hyperthyroidism. This is a great advantage since the validity of this method depends upon the assumption that starvation affects neither the uptake of iodide by the thyroid nor the rate of turnover of hormone in the tissues. It is quite unlikely that so brief a period of starvation would influence either of these processes.

If the excretion of organic iodine turns out to be negligible, equation 26 may also be used as a method for calculating the rate of secretion of the thyroid hormone: Solving equation 26 for H:

$$H = \frac{E_s}{(1 - U)} + F \qquad 29$$

and, if F is negligible:

$$H \cong \frac{E_s}{1 - U} \qquad 30$$

A second but much more cumbersome method for calculating F is based on the obvious fact that the rate of loss of labelled hormone from the thyroid gland

("biological decay") is dependent not only upon the excretion of iodide from hormone broken down in the tissues but also upon the loss of organic iodine from the body.

If sufficient time be allowed for equilibration after the administration of radioactive iodine, the specific activity of the hormone will be the same in the tissues as in the gland.[4] At that time:

$$24K_G = \frac{E^* + F^*}{Q_G^*} \qquad 31$$

Solving for F*:

$$F^* = 24K_G Q_G^* - E^* \qquad 32$$

Also, since F*, F, E* and (H − F) (1 − U) are all derived from the tissue hormone, F*/F and E*/(H − F) (1 − U) are both expressions for the specific activity and may be equated to each other:

$$\frac{F^*}{F} = \frac{E^*}{(H - F)(1 - U)}$$

or

$$\frac{F^*}{E^*} = \frac{F}{H(1 - U) - F(1 - U)} \qquad 33$$

Substituting in equation 33 the value for F* from equation 32:

$$\frac{24K_G Q_G^* - E^*}{E^*} = \frac{F}{H(1 - U) - F(1 - U)} \qquad 34$$

But from equation 15a,

$$H(1 - U) = UE \qquad 15a$$

Substituting this value in equation 34:

$$\frac{24K_G Q_G^* - E^*}{E^*} = \frac{F}{UE - F(1 - U)} \qquad 35$$

Solving for F:

$$F = \frac{UE(24K_G Q_G^* - E^*)}{24K_G Q_G^*(1 - U) + UE^*} \qquad 36$$

[4] This statement, which is repeated in Section VII-B, is sufficiently accurate for practical purposes, but it is not quite precise. The specific activity of hormone in the tissues will at first gradually increase as the specific activity in the gland steadily decreases (Figure 8). When the specific activity of hormone in the tissues attains its peak, for an instant it will exactly equal the specific activity of the hormone in the gland. Thereafter the specific activity in the tissues will begin to decline, but at first somewhat more slowly than does the specific activity in the gland. As equilibrium is approached, the ratio of specific activity in the tissues to specific activity in the gland will tend towards a constant value, but this asymptotic value will always be a little greater than unity. This is in accord with the general relationship described by Zilversmit *et al.* (210) for the specific activities of any radioactive compound and its immediate precursor. Equations based on equality of specific activity of hormone in thyroid and tissues are therefore *strictly* valid only when the specific activity of the hormone in the tissues is maximal.

This mode of calculation requires measurement of the uptake, the excretion of both stable and radioactive iodine, the biological decay and the quantity of radioactive iodine remaining in the thyroid gland. Were there no limit to the amount of radioactive iodine which can be given, all of these could be measured with considerable precision. However, in normal subjects the time which must elapse between the administration of a tracer dose and the attainment of equal specific activities in gland and tissues is probably too long to permit very accurate measurements of the daily excretion of radioactive iodine in the urine. However, the method might be useful in patients with hyperthyroidism in whom equal specific activities in tissues and gland should be attained sooner, and to whom it may be permissible to administer larger doses of radioactive iodine.

Unfortunately, the available data permit only a very crude estimate of the quantity of iodine excreted daily in organic form. The reviewer will hazard a guess that in the normal human it amounts to no more than 10 per cent of the iodine secreted as hormone. For purposes of illustration, 6 microgm. per day will be taken arbitrarily as the sum of urinary and fecal excretion of organic iodine in euthyroid subjects.

Comparatively little is known about pathological variations in the excretion of organic iodine. Kydd and Man (87) have found abnormally high concentrations of protein-bound iodine in the serum of patients with infectious hepatitis during the first four weeks of illness. They suggest that during the early phase of the disease, severe hepatic damage impairs the ability of the liver to destroy and excrete thyroxine which therefore accumulates in the plasma. This explanation seems improbable. Even if it were supposed that most of the thyroxine secreted is normally metabolized or excreted by the liver, the anterior pituitary, by decreasing its secretion of thyrotropic hormone, should easily be able to prevent any significant rise in the plasma concentration of thyroxine (59).

Peters and Man (131) found small quantities of protein-bound iodine in the urine of patients with nephrosis. They concluded that the loss of hormone was not sufficient to explain the low concentrations of protein-bound iodine in the serum of nephrotic subjects. This conclusion is also supported by Recant (153). However, to the extent that abnormal quantities of hormone are excreted in patients with proteinuria, there must be a compensatory increase in the activity of the thyroid gland for maintenance of a normal supply of hormone to the tissues. This may explain the unusually rapid and extensive uptake of radioactive iodine which was observed in some of the nephrotic subjects studied by Recant (see Figure 12).

E. *The Blood Flow through the Thyroid Gland:* In proportion to its weight, the normal thyroid gland is more richly supplied with blood than any other organ of the body except the lungs. In animals the flow has been estimated at 5.6 ml. per gram of tissue per minute (15), while in man, Means (107) estimates the flow at 4 ml. per gram of tissue per minute. Means also quotes a prescient remark by King (85) who, in 1836, said of the thyroid gland that, "Its nourishment does not seem to be the main intention of its vascular supply." It is now abundantly clear that "the main intention of its vascular supply" is to make up for the ex-

ceedingly minute *concentration* of inorganic iodide in the blood stream by presenting the gland with a *volume* of blood sufficiently large to provide the quantity of iodide needed daily for hormone manufacture.

The mean concentration of iodide ion in the plasma may be calculated by solving equation 4 for (I):

$$(I) = \frac{E}{1.44C_K} \qquad 4a$$

This method of calculating the plasma concentration of the iodide ion is far more accurate than direct chemical estimation. The quantity of iodide excreted in the urine and the renal clearance of iodide may both be measured with considerable precision, while the actual concentration of iodide in plasma is too small to be measured accurately even with the highly sensitive analytical methods now in use. Since the normal kidneys clear iodide from the blood stream at the rate of approximately 33⅓ ml. per minute, and since the normal daily excretion of iodide is of the order of 144 microgm., the normal mean concentration of iodide ion in the plasma must be about 3 microgm. per liter. Hence, to obtain its daily requirement of 70 microgm., even the normal thyroid gland must extract the iodide from about 23 liters of plasma or 27 liters of whole blood each day.

Unfortunately, there seems to be a dearth of quantitative information on the maximum blood flow through the thyroid gland when it becomes hyperactive. It is common knowledge that the thyroid gland in Graves' disease and in hyperplastic goiter due to iodine deficiency is exceedingly vascular. How much of this increased vascularity is due to a simple increase in the size of the gland and how much to an increase in the blood flow per gram of tissue is not known. However, if the normal blood flow is approximately 5 ml. per gram of tissue per minute it seems unlikely that it could increase more than three- or four-fold and still leave room for active thyroid parenchyma.

A simple method for calculating the thyroid blood flow, which should at least be applicable to animals, is described in the following section.

F. *The Efficiency of Extraction of Iodide from the Blood Stream:* Like the daily secretion of hormone, the efficiency of extraction of iodide ion from the blood stream by the thyroid gland cannot be directly measured in the intact human being. However, it should be relatively simple to measure it in anesthetized animals or indeed in patients during operations on the thyroid gland. Samples of arterial blood flowing to, and venous blood flowing from, the gland could be collected after the administration of a tracer dose of radioactive iodine. The arteriovenous difference in the concentration of radioactive iodine would provide a direct measure of the efficiency of extraction:

$$P = \frac{(I)^*_A - (I)^*_{TV}}{(I)^*_A} \qquad 37$$

If time enough were allowed for the radioactive iodide to approach distribution equilibrium, venous blood from another region could be substituted for arterial blood.

Apparently Pochin (137) is the only investigator who has used this method. In six patients undergoing subtotal thyroidectomy for non-toxic adenoma the concentration of radioactive iodide in blood from a vein draining the more normal thyroid lobe was compared with the concentration in blood from an arm vein. The mean extraction efficiency was 0.2.

This technique could also be employed for the calculation of the thyroid blood flow. Solving equation 1 for ϕ:

$$\phi = \frac{1.19C_G}{M_G P} \qquad 1a$$

It is obvious that:

$$\frac{C_G}{C_K} = \frac{U}{1 - U} \qquad 38$$

Hence:

$$C_G = \frac{UC_K}{1 - U} \qquad 39$$

Substituting this value for C_G in equation 1a:

$$\phi = \frac{1.19UC_K}{(1 - U)PM_G} \qquad 40$$

Since U and C_K are easily measured, and since P can be calculated by equation 37, equation 40 provides a means of studying the total thyroid blood flow, ϕ M_G, under various conditions. If the thyroid gland were then removed and weighed, the blood flow per gram of gland per minute, ϕ, could also be calculated. Assuming normal values for the thyroid clearance and the weight of the thyroid gland, Pochin estimated that the mean blood flow in the six patients whom he studied was 6 ml. per gram of thyroid per minute.

An estimate of the efficiency of extraction may be derived from equation 8. Solving this equation for P,

$$P = \frac{1.19C_K H}{\phi M_G (In - F)} \qquad 8a$$

Normal values for all of the factors in this equation have already been estimated with the exception of M_G, the mass of the normal thyroid gland. For this we may accept the value of 20 gm. given by Means (107). Substituting these values in equation 8a:

$$P = \frac{(1.19)(33\ 1/3)(70)}{(5)(20)(150-6)} = 0.19$$

This is almost identical with the mean value given by Pochin. It is evident, therefore, that even in regions of relative iodine abundance the normal thyroid gland must remove almost 20 per cent of the iodine from the blood which flows through it. Even if the efficiency could increase to 100 per cent in response to a decreased iodine supply or an increased secretion of hormone, the clearance of iodide from

the blood stream by the thyroid gland would increase only about five-fold. The ability of the extraction efficiency to adapt itself to demands for *increased* thyroid function is therefore quite limited. On the other hand, the extraction efficiency can, and undoubtedly does *decrease* almost without limit in response to an excessive supply of iodide (see Section X, B).

The efficiency of the thyroid gland in extracting iodide from the blood stream is dependent in part upon its ability to maintain a concentration gradient of the iodide ion between gland and blood stream, and in part upon its ability to oxidize inorganic iodide and incorporate it rapidly into tyrosine. Since both of these mechanisms may be specifically impaired by certain drugs, further discussion of them will be deferred to the section on the influence of pharmacological agents on iodine metabolism and thyroid function.

VII. FACTORS WHICH INFLUENCE THE DISTRIBUTION OF RADIOACTIVE IODINE

Thus far the discussion has centered about iodine metabolism and thyroid function at equilibrium, and simple algebraic equations have sufficed. But the equilibrium is a *dynamic* equilibrium and for the study of its dynamics it is necessary to consider the distribution of a tracer dose of radioactive iodine as it traverses the various metabolic pathways leading to and from the several compartments. Mathematical treatment of the behavior of radioactive iodine within the body therefore requires the use of differential equations to describe the rate of change of the quantity of radioactive iodine in each compartment with the passage of time after the administration of a tracer dose. This rate of change will depend upon the difference between the rate at which radioactive iodine is entering the compartment and the rate at which it is leaving the compartment. Hence at any time after a tracer dose:

$$\frac{dQ_I^*}{dt} = K_{BI}\,Q_B^* - (K_{IE} + K_{IG})Q_I^* \qquad 41$$

$$\frac{dQ_G^*}{dt} = K_{IG}\,Q_I^* - K_{GB}\,Q_G^* \qquad 42$$

$$\frac{dQ_B^*}{dt} = K_{GB}\,Q_G^* - (K_{BI} + K_{BF})Q_B^* \qquad 43$$

The initial conditions assumed (Section III, B, 3) being:

$$\text{When } t = 0,\ Q_I^* = 1,\ Q_G^* = 0,\ Q_B^* = 0$$

Since equations 41, 42 and 43 are interdependent, a rigorous mathematical description of the distribution of radioactive iodine would require the simultaneous solution of all three equations which is by no means easy. The reader is referred to the paper by Oddie (126) for a discussion of some of the complexities involved. Of the three equations, only the first two are easily applied. During the first few hours after the administration of a tracer dose, the distribution will depend chiefly on K_{IE} and K_{IG} since, in proportion to these, all of the other rate constants are usually small enough to be neglected. Hence:

$$\frac{dQ_I^*}{dt} \cong -(K_{IE} + K_{IG})Q_I^* \qquad 41a$$

and

$$\frac{dQ_G^*}{dt} \cong K_{IG}\, Q_I^* \qquad 42a$$

Although Brownell (18) has suggested an approximate method for the calculation of Q_B^* from the various rate constants, it involves the assumption that the loss of labelled hormone from the gland is governed throughout by K_G. This assumption is not supported by the observed behavior of radioactivity in the gland (see Section IX, B).

Thus far, the problem of the distribution of radioactive iodine has been discussed as though the rate constants were known, and the quantities of radioactive iodine in each compartment were to be calculated from them. In practice, a mathematical analysis would be most useful for calculating the rate constants from serial observations of the quantities. In view of the difficulty of rigorous mathematical treatment, Brownell's proposal that an analogue computer be used in the analysis of data from tracer experiments is attractive (18).

The dynamic behavior of radioactive iodine will obviously be affected by all of the factors discussed in previous sections. In addition, however, it will be affected by a group of factors which have no direct influence on thyroid activity. The factors hitherto discussed are for the most part represented by the arrows in Figure 2. However, rates of transfer of radioactive iodine are equally dependent upon the sizes of the cubes in Figure 2. At equilibrium, the rate constant of transfer from a compartment along a given pathway is equal to the quantity of iodine traversing that pathway per hour divided by the quantity of iodine present in the compartment. The quantities of iodine contained by each compartment must now be discussed.

A. *The Iodide Compartment:* The total quantity of iodide ion in the body is the product of the iodide concentration in the plasma and the volume of distribution of the iodide ion.

$$Q_I = (I)V_I \qquad 44$$

It has already been pointed out that the most satisfactory method for calculating the iodide concentration is by the use of equation 4a:

$$(I) = \frac{E}{1.44C_K} \qquad 4a$$

Although it is possible to calculate the volume of distribution from equation 11, this is likely to be untrustworthy, since the theoretical value for the initial concentration of radioactive iodine in the plasma must be obtained by extrapolation of a logarithmic plot of observed plasma concentrations to zero time. Because of the time needed for absorption and distribution of the radioactive iodine, the extrapolated value is likely to be in error, particularly when the radioactive iodine is administered by mouth. It is therefore preferable to solve equa-

tion 9 for V_I and calculate the volume of distribution from the rate constant of excretion and from the renal clearance:

$$V_I = \frac{6C_K}{100K_{IE}} \qquad 45$$

The calculation of C_K has already been discussed. K_{IE} may be calculated from equation 10, provided that $K_{IE} + K_{IG}$ can be measured.
The integral of equation 41a is:

$$Q^*_{I_t} = Q^*_{I_0} e^{-(K_{IE} + K_{IG})t} \qquad 46$$

Hence

$$\ln Q^*_{I_t} = \ln Q^*_{I_0} - (K_{IE} + K_{IG})t \qquad 47$$

Solving for $K_{IE} + K_{IG}$:

$$K_{IE} + K_{IG} = \frac{\ln Q^*_{I_0} - \ln Q^*_{I_t}}{t} \qquad 48$$

It is evident, therefore, that $K_{IE} + K_{IG}$ is simply the slope of the straight line obtained when the natural logarithm of the quantity of radioactivity remaining in the iodide compartment is plotted against time. If the volume of distribution remains constant, the concentrations of radioactive iodide may be used in equation 48 instead of the total quantities.

Keating and his collaborators have pointed out that the rate of disappearance of radioactive iodine from the blood stream ($K_{IE} + K_{IG}$) may be estimated from its rate of appearance in the urine, its rate of collection by the thyroid gland, or its rate of disappearance from any indifferent region of the body such as the thigh, as well as from the rate of decrease of its concentration in the plasma (95).

Keating and Albert (80) have calculated that the volume of distribution of inorganic iodide in six euthyroid subjects was 26.0 ± 3.5 liters or 35 per cent of the body weight. Similar values were found in six patients with myxedema and three with adenomatous goiter without hyperthyroidism, the weighted mean for the whole group of 15 patients without hyperthyroidism being 24 liters or 34 per cent of the body weight. In 16 patients with exophthalmic goiter the apparent volume of distribution was somewhat larger, averaging 31.2 liters or 53 per cent of the body weight.

Myant and his collaborators (119) proposed a somewhat different method for the calculation of the volume of distribution. By subtracting from the administered dose the quantities of radioactive iodine which had already been accumulated by the thyroid gland and excreted in the urine, they obtained the quantity remaining in the body and presumably distributed throughout the iodide compartment. The amount remaining, divided by the concentration of radioactive iodide in the plasma gave the volume of distribution. At any given moment (prior to the appearance of labelled hormone in the blood stream):

$$V_I = \frac{1 - (Q^*_{G_t} + Q^*_{E_t})}{(I)^*_t} \qquad 49$$

The mean volume of distribution of iodide calculated in this manner in 14 healthy normal subjects was 18 liters, or 28 per cent of the body weight, at 1 hour after the administration of radioactive iodine intravenously. However, the iodide space appeared to increase further with the passage of time so that at 6 hours it was approximately 25 liters or 39 per cent of the body weight. The latter estimates agree well with those of Keating. The authors discuss a number of possible explanations for the apparent slow increase in the volume of distribution of iodide after the first two hours. In another paper Myant and his collaborators (123) comment as follows, "It seems reasonable to assume, therefore, that the radioiodide diffuses through a volume comparable with the body water after some hours." The reviewer does not feel that this conclusion is substantiated either by the data presented by Myant or by the more extensive studies of Keating's group.

If the expression for (I) of equation 4a and the expression for V_I of equation 45 be substituted in equation 44, the following very simple equation for the mean quantity of iodide in the iodide compartment is obtained.

$$Q_I = \frac{E}{24K_{IE}} \qquad 50$$

As a matter of fact, equation 50, and the analogous equations to be introduced later, need not be derived in this manner, since they are simply algebraic expressions of the definition of a rate constant at equilibrium. A mean normal value for K_{IE} may be obtained from the exceedingly extensive data of Keating and his collaborators (81). Excluding patients with renal, cardiac or thyroid disorders, or with debilitating diseases, such as carcinoma or Addison's disease, there remains a group of 202 euthyroid subjects in which the mean renal excretion rate, K_{IE}, was 0.072 per hour. Substituting this value, and the estimate of 144 microgm. per day for the normal excretion of iodide, in equation 50, one obtains an estimate of 84 microgm. of iodide in the entire iodide compartment. If the mean iodide concentration is taken as 3 microgm. per liter, the volume of distribution by equation 44 would be approximately 28 liters, which is in good agreement with the estimates quoted above. For illustrative purposes, a value of 25 liters will be taken as the mean volume of distribution of the iodide ion and a value of 75 microgm. as the mean quantity of iodide ion within the body in normal man.

The volume of distribution of the iodide ion is considerably larger than the volume of distribution of chloride, bromide or thiocyanate. This does not necessarily indicate that the iodide ion can penetrate the cells of most tissues more readily than the other ions. Iodide concentrated in the thyroid gland and in the secretions of the salivary and gastric glands may well account for the larger volume of distribution. For example, if the concentration of iodide in gastric juice is 30 times the concentration in plasma, 100 ml. of gastric juice will contribute 3 liters to the volume of distribution of iodide. Furthermore, the thyroid gland may, under certain circumstances, contain more iodide ion than all of the rest of the body together (see Section IX, C).

Pathological expansion of the iodide compartment occurs in patients with

edema. Not only is the volume of distribution of the iodide ion increased, but there may also be an unusual delay in the attainment of distribution equilibrium when a tracer dose of radioactive iodine is given. Myant and his coworkers (119) have found that the concentration of radioactive iodide in ascitic fluid, or in edema fluid draining from Southey's tubes, increases rather slowly and does not become equal to the plasma concentration until five or six hours after the intravenous administration of a tracer dose of radioactive iodine. The assumption of instantaneous and even distribution of radioactive iodine throughout the iodide compartment is patently invalid in patients with large reservoirs of edema fluid.

B. *Organic Iodine in the Thyroid Gland:* After the administration of a tracer dose of radioactive iodine, the specific activity of hormone in the tissues will gradually approach the specific activity of hormone in the thyroid gland. When the specific activities have become equal:

$$\frac{Q_G^*}{Q_G} = \frac{(B)^*}{(B)} = \frac{E^*}{(1 - U)(H - F)} \qquad 51$$

Hence:

$$Q_G = \frac{Q_G^*(B)}{(B)^*} \qquad 52$$

and:

$$Q_G = \frac{Q_G^*(1 - U)(H - F)}{E^*} = \frac{Q_G^*[H(1 - U) - F(1 - U)]}{E^*} \qquad 53$$

but:

$$H(1 - U) = UE \qquad 15a$$

Therefore:

$$Q_G = \frac{Q_G^*[UE - F(1 - U)]}{E^*} \qquad 54$$

Or, if F is negligible:

$$Q_G = \frac{Q_G^* UE}{E^*} \qquad 55$$

According to equation 52 it should be possible to calculate the quantity of organic iodine in the thyroid gland from the specific activity of protein-bound iodine in the blood stream. However, with tracer doses of reasonable size, the specific activity is likely to be too low for equation 52 to be of any practical value. Equation 55 should be more useful since it may be possible to concentrate the radioactive iodide from an entire 24-hour sample of urine and measure it with some precision.

Another method based upon specific activity is advantageous since it provides larger quantities of radioactive iodine in the urine. From equation 23 it is evident that when equal specific activities of the hormone in thyroid and tissues have

been attained and synthesis of hormone is completely blocked:

$$\frac{Q_G^*}{Q_G} = \frac{H^*}{H} = \frac{E_{b1}^* - E^*}{E_{b1} - E} \qquad 56$$

Solving for Q_G:

$$Q_G = \frac{(E_{b1} - E)Q_G^*}{E_{b1}^* - E^*} \qquad 57$$

This method has actually been used to calculate the quantity of iodine in the thyroid glands of patients with goiter due to iodine deficiency (179). Equation 57 is valid even if the blocking agent accelerates the rate of loss of radioactive iodine from the thyroid gland (see Section VI, B, 3) since the specific activity should not be altered thereby.

A second mode of calculation is based not upon specific activity, but upon the rate at which labelled hormone is lost from the thyroid gland.
By definition:

$$Q_G = \frac{H}{24K_{GB}} \qquad 58$$

If the thyroid gland could be instantaneously and uniformly labelled with radioactive iodine, K_{GB}, the rate constant of transfer of hormone from the gland to the tissues, could theoretically be obtained from the *initial* rate of loss of radioactivity from the gland, *i.e.*, the rate of loss before appreciable quantities of radioactive iodine begin to return to the gland from breakdown of labelled hormone in the tissues. In fact, however, the initial accumulation of radioactive iodide is not instantaneous, and is likely to overlap somewhat with the return of iodide from hormone synthesized and secreted during the early stages of uptake. This mode of calculation will tend to underestimate the true value for K_{GB} and will therefore lead to an overestimate of the quantity of organic iodine in the thyroid.

An alternative method depends on measurement of the rate of loss of radioactivity from the thyroid when equality of specific activities has been attained in gland and tissues. The rate of loss will then no longer be proportional to H but rather to:

$$(1 - U)(H - F) + F = H(1 - U) + UF \qquad 59$$

and therefore:

$$Q_G = \frac{H(1 - U) + UF}{24K_G} \qquad 60$$

Where K_G is the rate constant for the *net* loss of radioactivity from the gland. But from equation 15a:

$$H(1 - U) = UE \qquad 15a$$

Hence:

$$Q_G = \frac{U(E + F)}{24K_G} \qquad 61$$

and if F is negligible:

$$Q_G = \frac{UE}{24K_G} \qquad 62$$

As far as the reviewer is aware, no one has attempted to apply this method for the calculation of the quantity of organic iodine in the thyroid gland. However, the method may be illustrated by using the data of Burns and his coworkers

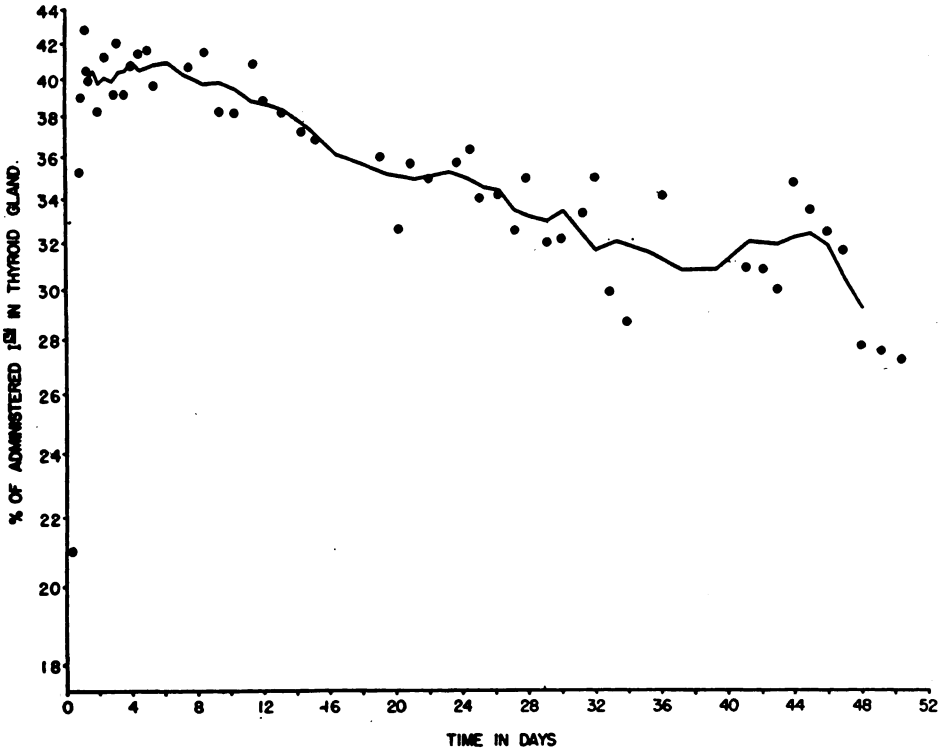

FIG. 4. The biological decay of radioactive iodine in the thyroid gland of a euthyroid subject studied by Burns *et al.* (21 (Subject No. 1)). The dose of radioactive iodide given at time zero was 100 microcuries. The per cent of the dose remaining in the thyroid has been corrected for physical decay and is plotted on a logarithmic scale against time. The solid line represents a five-point moving average. Note the very slow rate at which radioactive iodine is lost from the gland.

(21) who measured the loss of radioactivity from the thyroid glands of two normal subjects during a period of 50 days. Through the kindness of Professor W. A. Fish, the data from one of these subjects are reproduced in Figure 4. In this subject the biological half-life was calculated to be 94 days (21). The value of K_G calculated from this figure is 0.000306. The uptake was 0.417. Using these figures, and the assumed values of 144 microgm. per day for E and 6 microgm. per day for F, the quantity of organic iodine in the thyroid gland calculated by equation 61 is approximately 8,500 microgm., a value very close to the normal mean.

In his monograph, McClendon (101) has tabulated the results of two extensive series of analyses in which the iodine content of the normal human thyroid was determined. In 30 glands analyzed by Remington, McClendon and their coworkers in Charleston, South Carolina, the average quantity of iodine per gland was 8,530 microgm. In 52 glands analyzed by Leland and Foster in New York, the average iodine content per gland was 7,444 microgm. The weighted mean of these two series is 7,840 microgm. We may conveniently take 8,000 microgm. as the mean quantity of organic iodine in the normal human thyroid.

McClendon's tabulation indicates that even in normal individuals considerable variation in the iodine content of the thyroid may be expected. Moreover, the administration of iodide will tend to increase the quantity of stored hormone. In Graves' disease and in iodine deficiency the concentration of hormone in the gland may be very greatly reduced although the total quantity may be decreased only moderately because of the increase in the size of the gland. These variations in iodine content will profoundly influence the rate at which radioactive iodine is turned over in the thyroid.

C. *Organic Iodine in Extrathyroidal Tissues:* Surprisingly little attention has been given to the influence of organic iodine in the peripheral tissues on the behavior of radioactive iodine. The methods which will now be described for the calculation of the quantity of extrathyroidal hormone are entirely analogous to those already used for calculation of the quantities of iodine in the iodide compartment and in the thyroid gland.

It is obvious that:

$$Q_B = (B)V_B \qquad 63$$

The concentration of organic iodine in the plasma, (B), is easily measured by determining the concentration of protein-bound iodine. The mean normal value may be taken as 50 microgm. per liter. Unfortunately, however, there are very few estimates available of the volume of distribution of the thyroid hormone, and even these are derived from studies of the distribution of exogenous thyroxine, rather than endogenous hormone. It is generally agreed (2, 122, 153) that after intravenous injection the concentration of thyroxine in the plasma decreases at a moderately slow rate for the first six to twelve hours, and thereafter at a very slow rate. The initial decrease is presumably due to the diffusion of thyroxine from the blood stream to the tissues and the subsequent very gradual decrease is presumably due to the gradual breakdown and excretion of thyroxine. From the second portion of the curve for thyroxine in the plasma Albert and Keating (2) calculated that the volume of distribution of thyroxine was 11.1 liters, or 19.3 per cent of the body weight, in a cretin maintained in a euthyroid state with desiccated thyroid. This volume of distribution seems surprisingly small. In a similar study Myant and Pochin (122) investigated the distribution of racemic or levo-thyroxine labelled with radioactive iodine in six normal subjects. The mean volume of distribution was 13 per cent of the body weight at one hour, 22.8 per cent of the body weight at 6 hours, and 32.6 per cent of the body weight at 24 hours after intravenous administration. As nearly as may be judged from these figures, distribution was practically complete at 24 hours.

For an individual weighing 70 kgm. the volume of distribution would therefore be approximately 23 liters. Taking the concentration of organic iodine as 50 microgm. per liter, this would give a total of 1,150 microgm. of organic iodine in the extrathyroidal tissues.

A second mode of calculation makes use of an equation entirely analogous to equations 50 and 58. By definition:

$$Q_B = \frac{H}{24(K_{BI} + K_{BF})} \qquad 64$$

Theoretically, if the secretion of hormone from the thyroid gland could be abruptly eliminated, the rate constant of disappearance of hormone from the tissues, $K_{BI} + K_{BF}$, could be calculated from the rate at which the protein-bound iodine of the serum decreased. This cannot be done in the intact human being. Alternatively, the rate of decrease of protein-bound iodine could be measured after abrupt withdrawal of thyroid from patients with myxedema, but such studies have not been systematically performed. The reviewer is forced to base his estimate for the peripheral rate of turnover of thyroid hormone on fragmentary evidence which he has culled from his own records and those of others. It should be emphasized that not one of these bits of evidence could survive careful scrutiny. Taken together, they suggest that the normal biological half-life of hormone in the tissues is very roughly ten days, and that $K_{BI} + K_{BF}$ is about 0.0029. If the normal rate of hormone secretion be taken as 70 microgm. of thyroxine iodine per day, substitution of this value in equation 64 yields an estimate of approximately 1,000 microgm. of organic iodine in extrathyroidal tissues.

Trunnell and his collaborators (194) estimate that the biological[5] half-life of radioactive iodine (presumably organic) in the tissues is 13.3 days. This estimate was obtained from studies in which doses of radioactive iodine large enough to destroy thyroid function were administered, but the secretion of hormone was probably not eliminated abruptly. Indeed, for several days such large doses may accelerate the loss of organic iodine from the gland and much of this organic iodine may be thyroglobulin rather than thyroxine. Under these circumstances it is difficult to evaluate the significance of the half-life.

The "balance-sheet" method used by Myant (see Section VII, A) for calculating the volume of distribution of inorganic iodide could also be applied to the volume of distribution of organic iodine. At any time after completion of the initial collection and excretion of radioactive iodide:

$$V_B = \frac{1 - (Q^*_{G_t} + Q^*_{E_t} + Q^*_{F_t})}{(B)^*_t} \qquad 65$$

The accuracy of this method is limited by the precision with which Q^*_{Et}, Q^*_{Ft} and particularly $(B)^*_t$ can be measured after a tracer dose of radioactive iodine.

[5] Actually Trunnell *et al.* report that the "biologic half-life" is 5 days. By this they mean the total loss due to both radioactive decay (half-life 8 days) and loss from tissues (half-life 13.3 days). The reviewer feels that it is less confusing to reserve the term "biological half-life" for the purely biological process of turnover in the tissues.

However, the data of Goldsmith and his coworkers (52) suggest that the method might easily be used in patients with hyperthyroidism.

A direct estimate of the volume of distribution of organic iodine could be obtained from chemical analysis of serum and tissues removed from euthyroid humans at autopsy. Such studies do not appear to have been made. Carr (23) has recently completed a careful analysis of the distribution of protein-bound iodine in the tissues of normal dogs. The results indicate that the volume of distribution of the thyroid hormone is considerably larger than the volume of extracellular fluid. Rall and his coworkers (143) studied the distribution of radioactive iodine in the blood and tissues 56 hours after the oral administration of 63 millicuries of radioactive iodine to a patient with metastatic carcinoma of the thyroid. In most tissues such as bone marrow, brain, liver, kidneys, muscle, spleen and gastrointestinal tract the concentration of radioactive iodine which was precipitable with zinc hydroxide and therefore presumably organic, was equal to or greater than the concentration of precipitable iodine in the blood. This would imply a volume of distribution considerably higher than the estimates derived above. Again, these data must be interpreted cautiously because of the destructive action of a large dose of radioactive iodine on the thyroid gland.

In the absence of more exact information the reviewer estimates that the normal quantity of organic iodine in the extrathyroidal tissues is 1200 microgm., and that the volume of distribution of organic iodine in extrathyroidal tissues is 24 liters. These estimates are based largely upon the studies of Myant and Pochin. The normal rate of turnover of thyroid hormone in the tissues may now be calculated from equation 64:

$$Q_B = \frac{H}{24(K_{BI} + K_{BF})} \qquad 64$$

$$1200 = \frac{70}{24(K_{BI} + K_{BF})}$$

$$K_{BI} + K_{BF} = 0.00243$$

which corresponds to a half-life of 11.9 days. The probability that $K_{BI} + K_{BF}$ varies with the concentration of thyroid hormone will be discussed in Section XI, A.

In Table 3 are summarized all of the normal values which have been discussed in the preceding sections, together with other quantities which have been calculated from them. While most of the values are certainly of the correct order of magnitude, the values for V_B and F are highly tentative and may require drastic revision as new evidence accumulates. Perhaps the best that can be said of the tabulated figures is that they are internally consistent. For comparison the table also contains values which are probably typical of certain common alterations in thyroid function. These and certain other changes in thyroid activity will now be considered.

VIII. PHYSIOLOGICAL VARIATIONS IN IODINE METABOLISM AND THYROID FUNCTION

In comparison with most of its endocrine partners, the thyroid gland is remarkably phlegmatic. Its sole duty seems to be the maintenance of an optimal

TABLE 3

Illustrative values of the quantities discussed in the text

QUANTITY*	UNITS	CONDITION				
		Normal (Fig. 2)	Acute iodine deficiency (Fig. 5)	Chronic iodine deficiency (Fig. 6)	Thyrotoxicosis (Fig. 7)	Thyrotoxicosis. Acute block of hormone synthesis (Fig. 9)
H	microgm./day	70	70	70	597	597
In	microgm./day	150	15	15	250	250
E	microgm./day	144	53.1	9	199	796
F	microgm./day	6	6	6	51.1	51.1
Q_I	microgm.	75	27.8	4.69	103	664
Q_G	microgm.	8,000	8,000	3,000	5,500	4,310
Q_B	microgm.	1,200	1,200	1,200	3,360	3,360
V_I	liters	25	25	25	25	40
M_G	gm.	20	20	120	60	60
V_B	liters	24	24	24	24	24
(I)	microgm./l.	3.0	1.11	0.19	4.14	16.6
(G)†	microgm./kgm.	400,000	400,000	15,000	91,700	71,800
(B)	microgm./l.	50	50	50	140	140
C_G	ml./min.	16.2	16.2	259	100	0
C_K	ml./min.	33.3	33.3	33.3	33.3	33.3
U	proportion	0.327	0.327	0.886	0.750	0.000
1-U	proportion	0.673	0.673	0.114	0.250	1.000
K_{IG}	proportion/hr.	0.0389	0.0389	0.622	0.240	0.000
K_{IE}	proportion/hr.	0.0800	0.0800	0.0800	0.0800	0.0500
K_{GB}	proportion/hr.	0.000364	0.000364	0.000972	0.00452	0.00577
K_G	proportion/hr.	0.000255	‡	0.000185	0.00142	‡
K_{BI}	proportion/hr.	0.00222	0.00222	0.00222	0.00676	0.00676
K_{BF}	proportion/hr.	0.000208	0.000208	0.000208	0.000635	0.000635
Biol. ½ life of						
I^{131} in I	hrs.	5.8	5.8	1.0	2.2	13.9
I^{131} in G (initial)	days	79	79	30	6.4	5.0
I^{131} in G (final)	days	113	‡	156	20.3	‡
I^{131} in B	days	11.9	11.9	11.9	3.9	3.9

* For the definition of the symbols used in this table, see Section V.

† The concentration of organic iodine in the thyroid gland expressed as microgm. per kgm.

‡ These values cannot be calculated since the conditions assumed are not equilibrium conditions.

concentration of thyroid hormone in the tissues (145). Physiological variations in thyroid function occur for the most part only when an increased rate of utiliza-

tion of hormone in the tissues demands a compensatory increase in the rate of secretion or when the manufacture of a normal daily quantity of hormone is impeded.

A. *Sex:* There seems to be little difference in thyroid activity between the sexes as indicated by serum protein-bound iodine (148) or uptake of radioactive iodine (139).

B. *Age:* Except during pregnancy the concentration of hormonal iodine in the blood stream remains remarkably constant from birth to death. While there may be a downward trend with advancing age, the decrease is small (195). However, there is at least circumstantial evidence that the rate at which the tissues utilize thyroxine does not remain constant throughout the life cycle. In regions of iodine deficiency the increased incidence of goiter in children at puberty and particularly in adolescent girls has been abundantly documented (98, 101, 107). Although this strongly suggests an increase in the requirement for thyroid hormone during sexual maturation, objective proof is lacking. In old age, Perlmutter and Riggs (128) found a significant decrease in the rate at which the thyroid gland accumulates radioactive iodine. The decrease was most striking in females beyond the menopause and, judging from a small number of determinations, was not associated with any significant decline in the concentration of protein-bound iodine in the serum. The authors suggested that in senescence the activity of the thyroid gland decreases because of decreased peripheral utilization of the thyroid hormone. Unfortunately, these investigators depended solely upon the the *rate* of accumulation as measured by the "accumulation gradient". Even if there were no change in thyroid function, some decrease in the accumulation rate in elderly individuals might be expected because of the tendency for renal function to decline with advancing years (176). More significant is the report of Quimby and her collaborators (139) that the uptake of radioactive iodine decreases slightly in successive decades. Thyroid function in adolescence and senility should be reinvestigated using the methods for the calculation of the daily secretion of thyroid hormone which have been described.

C. *Pregnancy:* So far as is known, pregnancy is the only physiological state regularly accompanied by an increase in the concentration of thyroid hormone in the blood stream. The protein-bound iodine of the serum commonly falls in the range of 7 to 10 microgm. per cent, values which would be characteristic of mild hyperthyroidism in non-pregnant individuals (74, 96, 132). But in pregnancy the increased concentration of hormone in the plasma seems to be necessary for the maintenance of a *normal* metabolic state since it is fully established by the sixteenth week, long before the occurrence of the hypermetabolism which is characteristic of the third trimester. Danowski has mentioned the possibility that the increased concentration of protein-bound hormone might be explained by an increase in the protein groups responsible for binding thyroxine (36). If this factor alone were responsible, thyroid function itself should be normal. Yet there is good evidence that in pregnancy as in adolescence, hyperplasia of the thyroid gland is prone to occur, especially in regions of iodine deficiency. Furthermore, an increase in the avidity of the thyroid gland for radioactive iodine has

been noted in pregnant rabbits (75). The reaction of the thyroid gland to pregnancy awaits clarification by further studies.

D. *Exposure to Cold:* In small laboratory animals such as the rat exposure to cold produces thyroid enlargement and increases the quantity of exogenous thyroid hormone required for the prevention of goiter due to the administration of thiouracil derivatives. Uotila (196) found that section of the pituitary stalk would prevent the hypertrophy of the thyroid due to cold exposure but not the compensatory hypertrophy due to subtotal thyroidectomy. This would suggest that the thyroid enlargement is due to primary stimulation of the anterior pituitary gland. However, the recent demonstration that the protein-bound iodine in the serum not only fails to rise but may actually decrease slightly when rats are exposed to cold strongly suggests that the anterior pituitary is stimulated by an increased rate of withdrawal of hormone from the blood stream, and that the thyroid hyperplasia is no more than a compensatory response to an increase in the rate of turnover of the thyroid hormone in the tissues (44, 145). In man, chronic exposure to cold in the Arctic also caused no increase, and possibly a slight decrease, in protein-bound iodine, without any significant change in the basal rate of metabolism (54). While these results provide no evidence for an effect of a cold environment on thyroid function in man, they by no means exclude the possibility that, during exposure to cold, the tissues may require an increase in the supply of thyroid hormone for maintenance of a *normal* metabolic rate.

E. *Other Stresses:* Violent exercise (148), inanition due to anorexia nervosa (131), a low protein intake (Kempner rice diet) (34), and a prolonged oversupply of adrenomedullary hormone in a patient with pheochromocytoma (154) are all apparently without effect on thyroid function as measured by the concentration of protein-bound iodine in the serum. In addition the accumulation of radioactive iodine is normal in anorexia nervosa (81, 200). However, the profound effect which adrenocortical hormones may have upon thyroid function (1, 14, 46, 76, 113, 130) suggests that the possible influence of stresses strong enough to stimulate the adrenal cortex (17) should be carefully investigated in man.

F. *Iodine Deficiency:* The effect of a decreased intake of iodine upon iodine metabolism and thyroid function illuminates many of the concepts developed in the preceding sections. The following brief description is illustrated by Figures 5 and 6, which should be compared with Figure 2.

Consider a euthyroid individual living in a region of iodine abundance, and in equilibrium when his mean intake of iodide is 150 microgm. per day (Figure 2). Immediately upon taking up residence in a region of severe iodine deficiency he will be subjected to the state of negative iodine balance illustrated by Figure 5. If the daily intake of iodine is reduced from 150 to 15 microgm. the concentration of iodide in the iodide compartment will rapidly decrease to a level which, as in starvation, will be determined largely by the quantity of iodide returned from breakdown of hormone in the tissues (see Section VI, D, 2). Since the renal clearance of iodide does not change (179), iodide excretion will exceed intake and the thyroid gland will enter a long period of attrition during which its stores of hor-

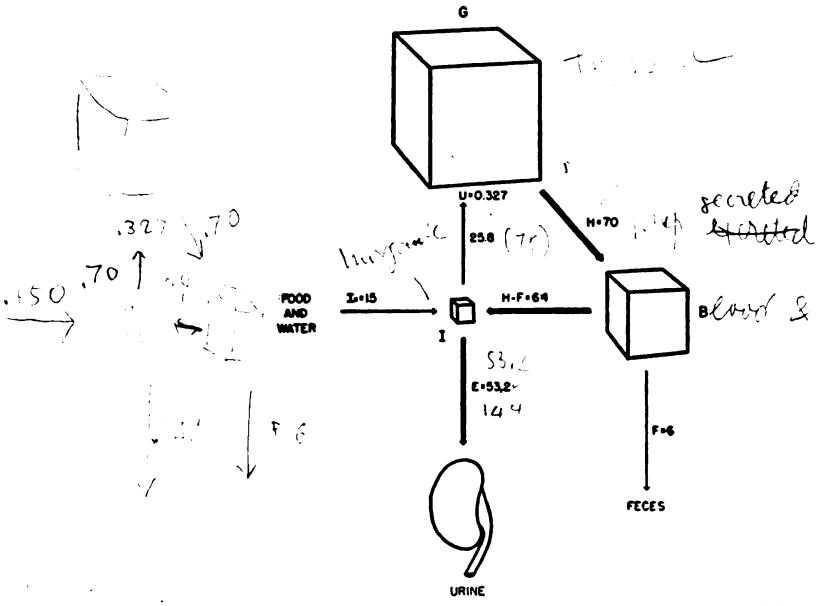

FIG. 5. A diagram of iodine metabolism in the euthyroid subject of Figure 2 soon after he has moved to a region of iodine deficiency (Table 3, column 4). The construction of the diagram has been explained in the legend of Figure 2. Note the decrease in size of compartment I.

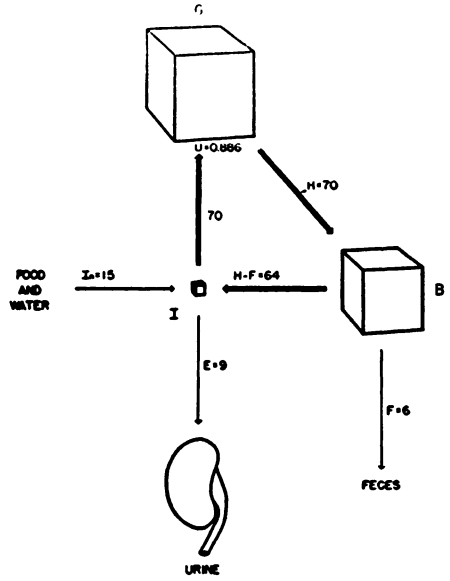

FIG. 6. A diagram of iodine metabolism in the euthyroid subject of Figure 5 after iodine balance has been attained in the face of a reduced intake of iodide (Table 3, column 5). The construction of the diagram has been explained in the legend of Figure 2.

Note that compartment G has become smaller, and that compartment I has shrunk to minute size. The importance of reutilization of iodide released from the breakdown of hormone in compartment B is evident from the figure.

mone gradually approach exhaustion. The duration of this period will depend upon the difference between iodide intake and excretion, the rate at which hormone is being secreted, and the quantity of preformed hormone. The state of imbalance illustrated in Figure 5 could theoretically continue for 181 days before the last reserves of stored hormone are exhausted. However, it is probable that as the concentration of organic iodine in the thyroid gland decreases, it becomes more and more difficult[6] for the thyroid to mobilize the quantity of hormone which it must secrete daily in order to maintain a normal concentration in the blood stream. Experiments in animals suggest that this may first become prominent when the concentration falls to about 0.1 per cent of the dry weight of the gland or about one half of the normal concentration (94). When the supply of hormone to the tissues decreases, the anterior pituitary gland increases its secretion of thyrotropic hormone. In consequence the thyroid gland becomes hyperplastic, the mass of active tissue increases, the blood supply enlarges, and the ability to accumulate iodide ion against a concentration gradient is enhanced. These compensatory reactions increase the rate at which the gland clears iodide from the blood stream (equation 1) and ultimately enable it to meet successfully the competition of the kidney for the available supply of iodide. The new equilibrium which is established is illustrated in Figure 6. The thyroid gland is now subsisting largely upon the iodide returned to the blood stream by breakdown of hormone in the tissues. Although the renal *clearance* of iodide remains constant, the total *quantity* of iodide lost in the urine is greatly diminished because the concentration of iodide ion in the iodide compartment has fallen to less than 0.2 microgm. per liter (see Table 3).

In Figure 6 it has been assumed for simplicity that complete compensation has been achieved in the face of a diminished iodide intake. However, theoretically compensation must fall somewhat short of its mark else the anterior pituitary would decrease its stimulus and the thyroid gland would tend to revert to normal, as indeed it does if the iodine deficiency is promptly corrected. Presumably the concentration of thyroid hormone perfusing the pituitary must remain somewhat low, yet among a large number of goiterous subjects studied in Argentina only a few had significantly decreased concentrations of protein-bound iodine in the serum (179). Probably also the very large goiters which are often caused by iodine deficiency are due to prolonged, rather than maximal, stimulation by thyrotropic hormone. Further increases in thyroid function can still be produced by the administration of exogenous thyrotropic hormone (179).

Astwood (8) has raised the interesting question of whether a decreased concentration of iodine in the thyroid gland may itself stimulate thyroid activity without the participation of the anterior pituitary. If this mechanism exists, it might of course contribute to the development of goiter, but it hardly seems necessary to invoke any such additional stimulus.

It should be emphasized that the simple cycle of events just described applies only to the initial stage of endemic goiter. Persistent stimulation by endogenous thyrotropic hormone seems to result in man, as it does in animals (114, 115), in

[6] A possible reason for the increasing difficulty is discussed in Section X-B.

the development of more or less discrete thyroid adenomas which are extraordinarily variable in histological structure. It is conceivable that some of these nodules may become autonomous and continue to function independently of thyrotropic stimulation. If the iodine deficiency is corrected, such nodules might feast upon the newly abundant iodide and secrete hormone without regard for bodily needs, even though the support of the anterior pituitary be withdrawn. This would explain the occurrence of "Jodbasedow", a phenomenon whose very existence has been questioned in recent years. Regardless of the mechanism involved, the reviewer feels that the transient increase in the incidence of hyperthyroidism when iodized salt was first introduced in the midwest (73, 84, 102, 103, 135) was not only very real but difficult to explain by anything except the increased supply of iodide.

The production of goiter by iodine deficiency is no longer hypothetical. Unless compensatory mechanisms fail, long continued ingestion of a diet markedly deficient in iodine inevitably causes enlargement of the thyroid gland, with an increase in blood supply sufficient to allow the daily collection of a normal total quantity of iodide despite a much reduced concentration in the blood stream. The only alternative would be to assume that a gland of normal size could accommodate a liter and a half of blood per minute and could extract the iodide from this torrent with normal efficiency. Since this alternative is obviously ridiculous, the recurring argument that iodine deficiency is not a sufficient cause of endemic goiter (55, 56, 57) is finally and utterly demolished. *In euthyroid persons with normal renal function goiter is an obligatory response to prolonged and severe iodine deficiency.* This categorical statement must not be interpreted to mean that iodine deficiency is the *sole* cause of goiter even in regions where the supply of iodine in food and water is minimal. Any other factor which impairs the efficiency of hormone synthesis or which increases the requirement for thyroid hormone will reinforce the goitrogenic effect of a diminished iodide intake.

G. *Other Dietary Factors:* Astwood and his coworkers have conducted a remarkable series of experiments on the thyroid-inhibiting properties of various foods (61). These investigations led to the isolation of an active goitrogenic agent, l-5-vinyl-2-thioöxazolidone, from various brassicaceous plants (9). Pharmacologically this naturally occurring goitrogen resembles the synthetic drugs of the thioamide series. The influence of diet on thyroid function and iodine metabolism has been competently reviewed by Greer (58).

IX. PATHOLOGICAL VARIATIONS IN IODINE METABOLISM AND THYROID FUNCTION

In this section the discussion will be limited to those types of thyroid disease which produce striking aberrations of iodine metabolism. Certain clinically important diseases of the thyroid such as cretinism in regions of endemic goiter, thyroiditis, and benign or malignant tumors of the thyroid gland will not be considered. Furthermore, the differences between diffuse hyperplasia and toxic adenoma will for the most part be disregarded.

A. *Hypothyroidism:* According to the model presented here, the complete

absence of functional thyroid tissue should reduce iodine metabolism to the relatively simple processes of absorption, distribution and excretion. In general this expectation is confirmed by the behavior of radioactive iodine in patients with severe myxedema (18, 175). Because the renal clearance of iodide tends to be reduced in patients with hypothyroidism (Table 1) the excretion of a tracer dose of radioactive iodine in the urine is somewhat delayed. But if urine specimens are accurately collected for three or more days, the total quantity recovered in the urine is usually 100 per cent of the administered dose within the error of measurement (18, 175). However, even in patients with severe myxedema Keating and his collaborators have often been unable to recover more than about 85 per cent of the administered dose (82). The significance of the "missing" iodine is quite obscure. Keating is inclined to ascribe it to retention by extrathyroidal tissues. This explanation is unattractive, since it would demand that a very considerable quantity of iodide be sequestered for a long period of time, either in the tissues as a whole or in specific sites. The rate constant of transfer of iodide from the iodide compartment to the sites of accumulation would have to be much larger than the rate constant of transfer in the reverse direction. At equilibrium, the total quantity of iodide in these putative sites of accumulation would therefore have to be greater than in the iodide compartment proper. Since there is no independent evidence for any such sequestration of iodide in relatively high concentration by extrathyroidal tissues, and since the majority of investigators have been able to account for practically 100 per cent of the administered dose, it seems likely that the "missing" iodide can best be explained by small, but consistently negative, errors in the collection of urine samples and in the measurement of radioactivity.

Keating's group has also demonstrated that, even in patients with myxedema, a small amount of radioactive iodine which is precipitable with zinc and therefore presumably organic appears in the blood stream. In one instance the maximum concentration of precipitable radioactive iodine was practically attained within a few hours of administration of the tracer dose, and then remained constant for approximately one week (104). This might possibly represent extrathyroidal synthesis of thyroxine, a process described by Chaikoff and his collaborators in animals (77, 116). But, since in patients with severe myxedema the concentration of protein-bound iodine in the blood stream as determined chemically is usually very close to zero, it is extremely doubtful that significant quantities of hormone are synthesized in man by extrathyroidal tissues. A more likely alternative is the possibility that even in a patient with no detectable accumulation of radioactivity in the neck, there may remain small bits of functional thyroid. Because of intense stimulation by thyrotropic hormone such remnants of thyroid tissue would be expected to secrete whatever hormone they can manufacture as rapidly as possible. This would account for the prompt but very limited appearance of organic radioactive iodine in the blood stream.

B. *Hyperthyroidism:* The changes in iodine metabolism characteristic of hyperthyroidism are illustrated in Figure 7. The primary derangement is the increased rate at which hormone is synthesized and secreted. In diffuse hyper-

plasia the increased turnover of hormone usually causes a marked reduction in the concentration of stored hormone, but because of the increase in the size of the gland the total quantity is often reduced only moderately. From a review of the literature, Gutman and his coworkers (68) concluded that the mean quantity of organic iodine in the thyroid gland in patients with untreated exophthalmic goiter was 5,500 microgm. They also demonstrated that after treatment with iodide the mean quantity was increased to 28,200 microgm.

In constructing Figure 7 the following assumptions have been made: 1) That the renal clearance of iodide remains normal in hyperthyroidism. This is probably true (see Table 1). 2) That the volume of distribution of the iodide ion is

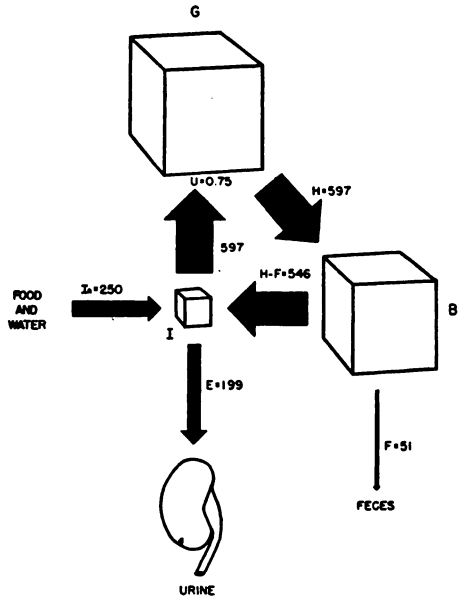

FIG. 7. A diagram of iodine metabolism in a patient with moderately severe thyrotoxicosis (Table 3, column 6). The construction of the diagram has been explained in the legend of Figure 2.

unchanged. Actually Keating and Albert (80) and Myant and his coworkers (119) have reported that the volume of distribution of iodide in hyperthyroidism is somewhat greater than normal. The cause of this increase is not clear. In part it may be due to the technical difficulties of estimating the volume of distribution of iodide when it is being removed from the blood stream very rapidly. It may also be due in part to the increased ability of the hyperactive thyroid gland to concentrate the iodide ion. However, the conversion of inorganic iodide to organically bound iodine is probably so rapid that even in Graves' disease the total quantity of iodide ion within the thyroid gland is small. 3) That the *proportion* of hormone lost from the body in organic form is the same as in the normal subject. While the information available suggests that the excretion of organic iodine may be greater than normal when the concentration of hormone

in the blood stream is elevated, there are no reliable measurements of this in man. 4) That the volume of distribution of organic iodine in the tissues is the same as in normal subjects. 5) That because of the increased food consumption characteristic of untreated hyperthyroidism the mean intake of iodide is increased from 150 to 250 microgm. per day.

In Figure 7 the uptake of radioactive iodine is assumed to be 0.75. This is somewhat higher than the mean value for the uptake of radioactive iodine at 24 hours in a large series of patients with hyperthyroidism studied by Keating *et al.* (81). It is somewhat lower than the mean value calculated from the quantity of radioactive iodine excreted in the urine as reported by Skanse (175). The concentration of protein-bound iodine in the plasma has been taken as 14 microgm. per cent. While the data assumed for illustration in Figure 7 are probably typical of moderately severe hyperthyroidism, it should be obvious that the actual values observed in any given patient may differ very widely from the ones chosen for illustration.

A comparison of iodine metabolism in the normal and thyrotoxic subjects illustrated by Figures 2 and 7 reveals certain striking and important differences. In the first place, although the daily secretion of hormone from the hyperactive thyroid gland has increased eight and one-half-fold, the concentration of hormone in the tissue compartment has increased by a little less than three-fold. This implies that the proportion of the hormone in the tissues which is turned over in unit time increases when the concentration of hormone increases. In the examples illustrated, tissue turnover, $K_{BI} + K_{BF}$, is about three times as rapid in the thyrotoxic patient as in the normal subject. A more detailed discussion of the quantitative relationship between the daily supply of hormone and the concentration of hormone in the plasma will be presented in Section XI, A.

In the second place it is of interest to compare the rate of loss of radioactivity from the thyroid gland immediately after accumulation is complete, with the rate of loss when the labeled hormone in the tissues has attained the same specific activity as the hormone in the gland. Solving equation 58 for the rate constant, K_{GB}:

$$K_{GB} = \frac{H}{24Q_G} \qquad 58a$$

Likewise:

$$K_G = \frac{H(1 - U) + UF}{24Q_G} \qquad 60a$$

Dividing equation 58a by equation 60a:

$$\frac{K_{GB}}{K_G} = \frac{H}{H(1 - U) + UF}$$

Solving for K_G:

$$K_G = K_{GB}\left[(1 - U) + \frac{UF}{H}\right] \qquad 66$$

If the values assumed for the normal subject are substituted in equation 66 it is found that the initial rate of loss of radioactive iodine from the gland is only about 1½ times the equilibrium rate of loss. In the thyrotoxic subject, however, the initial rate of loss is more than three times the equilibrium rate. It should be evident from equation 66 that *the true biological half-life of radioactive iodine in the thyroid gland cannot be calculated from the rate of loss of radioactivity immediately after collection is complete* (see Figure 8). Even in the normal subject studied by Burns and his collaborators (21) (see Figure 4) there is perhaps a

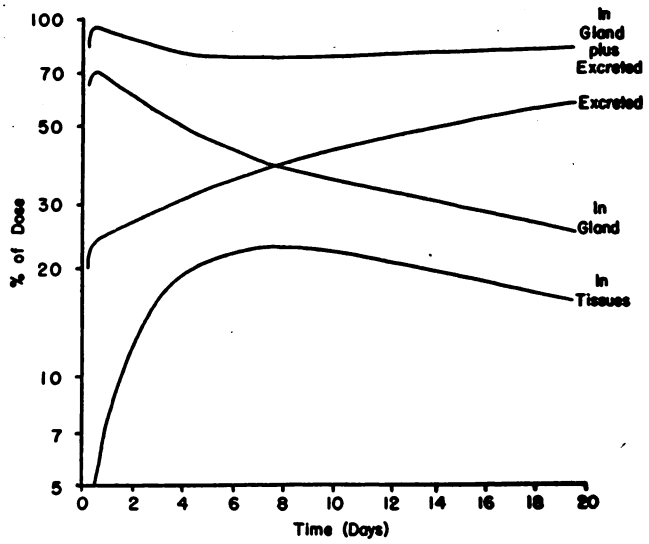

FIG. 8. The theoretical distribution of a tracer dose of radioactive iodine given to the thyrotoxic subject of Figure 7. The curves were not calculated mathematically but were obtained through the kindness of Dr. Gordon Brownell from an electrical analogue computer which he designed and constructed. The scale for per cent of dose is logarithmic.

Note the slow accumulation of radioactive iodine in compartment B, and the changing slope of the curve of decay of radioactive iodine in the thyroid gland. Note also that, as observed by Goldsmith and his collaborators (52), the sum of the radioactive iodine remaining in the thyroid and the radioactive iodine which has been excreted decreases during the first few days after administration of the tracer dose.

slight tendency for the radioactivity in the thyroid gland to decrease somewhat more rapidly during the first few days than later on. This tendency is much more clearly evident in curves plotted for several thyrotoxic subjects by Goldsmith and his co-workers (52).

In the third place, the rapid turnover of hormone in the gland is likely to result in an underestimate of the true uptake of radioactive iodine, as judged from *in vivo* measurements over the thyroid gland. This is particularly true when the measurements are made at 48 hours after the tracer dose (see Figure 11).

Finally, it should be noted that in thyrotoxicosis the hormone in the plasma and extrathyroidal tissues may constitute a very considerable portion of the total amount of hormone in the body. In the normal subject illustrated in Figure

2, the total quantity of extrathyroidal hormone is only 15 per cent of the quantity in the thyroid itself. In the thyrotoxic subject illustrated in Figure 7, the hormone in the plasma and tissues is 61 per cent of the hormone in the thyroid. In such a patient when the specific activity of the hormone in the tissues has attained the specific activity of hormone in the gland, nearly 40 per cent of the radioactivity remaining in the body would be extrathyroidal (see Figure 8). Goldsmith and his coworkers (52) could not account for all of the radioactivity administered when they summed the quantity remaining in the thyroid gland and the quantity which had been excreted in the urine. As nearly as can be judged from their rather complicated experiments, the discrepancy developed most rapidly during the first few days after the administration of the tracer dose and tended to remain constant thereafter. They concluded that, "15 to 40 per cent of the labeled iodine released from the thyroid in organic form is excreted by alternative routes". To the reviewer it seems much more likely that the majority of the "missing" iodine was distributed throughout the compartment of organic iodine in the tissues. This impression is strengthened by the very considerable proportion of the tracer dose which was present in the serum of some of their patients.

C. *Sporadic Cretinism:* In most patients with sporadic cretinism the thyroid gland is atrophic and iodide metabolism is like that in an adult patient with myxedema. Very rarely, sporadic cretins are enountered with tremendous enlargements of the thyroid gland due to an extraordinary derangement of iodide metabolism. In three goitrous siblings, Stanbury and Hedge (180) found that a large proportion of a tracer dose of radioactive iodine was concentrated within the thyroid gland. However, the behavior of the radioactive iodine was unusual in three respects. First, the peak concentration of radioactivity in the thyroid gland was attained within an hour or two after the oral administration of the tracer. Second, the amount of radioactivity in the thyroid declined very perceptibly during the next few hours. Third, the radioactive iodine was rapidly and almost completely discharged from the thyroid gland by the oral administration of potassium thiocyanate. Simultaneously, the concentration in other parts of the body increased. In two of the glands removed surgically the concentration of protein-bound iodine was only 10.4 and 15 microgm. per hundred grams. In contrast, the inorganic iodide was 29 and 173 microgm. per cent. The larger gland weighed 497 grams and probably contained a total of about 860 microgm. of inorganic iodide.

As the authors point out, the only logical explanation for these observations is that the patients were suffering from a hereditary defect which almost completely prevented the synthesis of thyroid hormone without impairing the ability of the thyroid tissue to maintain a high concentration gradient of inorganic iodide. The cause of the interference with synthesis is unknown, but the end result is remarkably similar to the block produced by drugs of the thioamide group. In essence these glands simply represented an enormous and completely useless extension of the iodide compartment. In the patient with the largest goiter, the iodide content of the thyroid tissue suggests that the volume of dis-

tribution of inorganic iodide must have been several times greater than the total volume of the body!

X. THE INFLUENCE OF DRUGS ON IODINE METABOLISM AND THYROID FUNCTION

Since there have been several recent reviews of the mechanism of action of antithyroid compounds, the discussion in this section will be limited to a cursory examination of the effect of certain drugs on iodine metabolism as represented by the simplified model. Dinitrophenol (49, 205), cortisone and adrenocorticotropin (1, 14, 46, 76, 113, 130), and blocking agents of the para-aminobenzene group (8) will not be considered, primarily because their mechanisms of action are poorly understood.

A. *Exogenous Thyroid Hormone:* Suppression of thyroid function by exogenous thyroid hormone has already been mentioned. The primary site of action is presumably the anterior pituitary gland which discontinues its secretion of thyrotropic hormone and reduces the activity of the thyroid gland to the minimum characteristic of hypophysectomy. In rats ablation of the pituitary diminishes the uptake of radioactive iodine (146), the turnover of thyroxine in the thyroid gland (147), and the ability of the thyroid to concentrate iodide ion from the blood stream (197) to about 10 per cent of normal. These observations are consistent with the earlier report of Chaikoff *et al.* (117) that hypophysectomy greatly retards the rate at which diiodotyrosine is converted to thyroxine. In man, Greer's study of the effect of thyroid on the uptake of radioactive iodine in euthyroid subjects also suggests that thyroid activity is very much depressed, but not completely abolished, when the pituitary is inhibited (59). Greer also found that much larger amounts of exogenous thyroid hormone were required to inhibit uptake in patients with hyperthyroidism (60). While a discussion of the role of thyrotropin in thyrotoxicosis is beyond the scope of this paper, the ability of tetrabromthyronine to cause a decrease in the concentration of protein-bound iodine in the serum of patients with Graves' disease suggests that in this type of hyperthyroidism the thyroid gland may indeed be under pituitary control, and may be inhibited by exogenous hormone (92). There are two obvious advantages in using a brominated or chlorinated thyronine rather than thyroxine itself in studies of this sort. First, any inhibitory effects cannot be ascribed to the action of iodide ion derived from breakdown of the exogenous hormone. Second, since the exogenous hormone does not contain iodine, a reduction in the concentration of protein-bound iodine in the serum may be taken as an indication that the production of endogenous hormone has decreased. It is to be hoped that future studies with tetrabromthyronine or tetrachlorthyronine will provide additional quantitative information concerning the inhibitory effect of exogenous hormone in patients with thyrotoxicosis.

Besides inhibiting the secretion of thyrotropic hormone, exogenous hormone may also have a direct inhibitory effect on the thyroid gland itself (33, 151). However, in man this direct effect upon the thyroid is probably rather unimportant (129).

B. *Iodide:* The effects of an increased supply of iodide on the thyroid gland are exceedingly complex and poorly understood. In the *normal* subject the administration of small amounts of iodide such as occur in iodized salt will for a time produce a non-equilibrium state which is precisely the reverse of iodide deficiency. The rate of manufacture of the thyroid hormone exceeds the rate of secretion and the total quantity of hormone stored in the gland therefore increases. Eventually, however, a new equilibrium is attained so that the uptake of iodide again balances the secretion of thyroxine. It is not entirely clear by what mechanism the uptake of iodide is decreased. It would seem logical to suppose that as the concentration of hormone in the colloid rises it becomes easier and easier for the thyroid cells to secrete hormone into the blood stream. De Robertis (42) has suggested that, before being secreted, thyroxine is released from thyroglobulin by a proteolytic enzyme whose activity is controlled by the thyrotropic hormone. If it be assumed that each unit of thyrotropic hormone promotes the proteolysis of a certain quantum of thyroglobulin, the amount of thyroxine thereby made available for secretion would depend upon the concentration of thyroxine in the thyroglobulin. When the concentration increases, the quantity of thyrotropic hormone which formerly just sufficed to stimulate the secretion of the normal daily supply of thyroid hormone now causes the secretion of a slight excess. This would tend to inhibit the secretion of thyrotropic hormone from the pituitary. In consequence, thyroid activity would decrease towards the level characteristic of hypophysectomy. The rate of uptake and turnover of iodine would therefore decline until the concentration of hormone in the blood stream returned to normal.

While this hypothesis offers a satisfactory explanation for the eventual inhibitory effect of a small increase in the daily supply of iodide, it can scarcely explain the achievement of equilibrium when several *grams* of iodide are administered daily. If the effects of hypophysectomy in man are at all comparable to those in the rat, the total withdrawal of hypophysial support should not decrease thyroid activity to less than about 10 per cent of normal. Yet to maintain equilibrium in the face of a continued intake of 1 gram of iodide per day, the uptake of iodide must be reduced to about 0.00007! Somehow the gland effects this all but complete suppression of uptake, and still manages to maintain its normal rate of secretion. It might be postulated that without any thyrotropic stimulation the residual activity of the thyroid gland might still be just sufficient to maintain normal synthesis and secretion of hormone as long as an optimal concentration of iodide was being supplied to the enzyme system concerned with synthesis. Greater increases in iodide supply could not further increase hormone synthesis since the synthetic mechanism would already be working at its maximal capacity. There is at present no acceptable evidence to support this hypothesis. Furthermore, it would imply that hypothyroidism secondary to pituitary failure could be treated by the administration of iodide. This seems unlikely, although so far as the reviewer is aware it has not actually been tried.

The acute effects of large doses of iodide in normal animals and humans have been more extensively studied. Wolff and Chaikoff (202) described a prompt

and virtually complete suppression of hormone synthesis. However, the inhibition of organic binding of iodine could not be prolonged beyond about two days, even when high concentrations of iodide were maintained in the plasma (203). In man a similar acute effect of doses of iodide sufficient to raise the plasma concentration to between 6 and 12 microgm. per cent was observed by Stanley (181). However, as Keating *et al.* point out (28), it is impossible solely by *in vivo* observations to prove conclusively that organic binding of iodide has been completely inhibited.

In patients with *Graves' disease* the daily administration of iodide in doses of about 1 mgm. usually produces no obvious change in the severity of the disease. Occasionally, however, such doses may provoke a striking increase in toxicity (190, 192). This suggests that in thyrotoxicosis the rate of secretion of thyroid hormone is sometimes limited by the quantity of iodide available. An increase in the supply of iodide removes this limitation, the manufacture and release of hormone are accelerated, and an exacerbation results.

In contrast, the daily administration of iodide in doses of 6 mgm. or more produces a rapid amelioration of signs and symptoms. In patients with exophthalmic goiter the response is obtained with such regularity that it may be used as a diagnostic test. In patients with toxic nodular goiter, the response to large doses of iodide is much less dramatic and may be entirely absent. The improvement produced by iodide in Graves' disease, though rapid, is usually incomplete. In some patients the improvement may be sustained for long periods if the administration of iodide is continued. In others, the disease may increase in severity even though iodide medication is faithfully maintained. If iodide be withdrawn, a prompt exacerbation is likely to occur, an exacerbation which is sometimes alarmingly severe, probably because the gland has made good its opportunity to store large quantities of thyroid hormone during the administration of iodide (26, 100).

The response to iodide is certainly due to a very rapid decrease in the rate at which hormone is secreted, but the reason for this decrease in secretion remains obscure. As in normal subjects, the acute administration of large doses of iodide to patients with thyrotoxicosis produces a prompt but presumably transient decrease in the organic binding of iodine by the thyroid (181). The blood concentration required for this inhibition is smaller than in the normal subject. Since the hyperplastic thyroid gland is able to maintain a much higher concentration gradient of iodide ion than the normal gland, it has been suggested that the inhibitory effect depends not on the concentration of iodide in the blood stream, but rather on the concentration of iodide within the thyroid gland (140, 181). Childs and his collaborators studied the effects of graded doses of carrier iodide on the accumulation of radioactive iodine (28). A dose of 0.1 mgm. produced no change in uptake. When the dose was progressively increased to 10 mgm. the proportion of the iodine utilized for hormone synthesis gradually decreased. Iodide still accumulated in the thyroid but apparently remained largely in the inorganic form since it tended to decrease rapidly as the kidneys excreted iodide from the body, and could readily be discharged by the administration of thiocya-

nate. Finally, even the iodide concentrating mechanism was saturated by doses of 500 mgm. or more. These observations show that the hyperplastic thyroid gland may actually accumulate much more iodide ion than it can utilize for the synthesis of hormone. But the decrease in the *proportion* of the available iodide which is used for manufacture of thyroxine cannot explain the iodide response in Graves' disease. Actually, during treatment with iodide the *absolute amount* of hormone stored in the thyroid gland and available for secretion is greatly increased. Further discussion of the confusing and contradictory observations and hypotheses concerning the inhibitory effect of iodide in exophthalmic goiter would serve no useful purpose. An excellent summary of the topic has been presented by Pitt-Rivers (133).

An additional perplexing effect of large doses of iodide is their ability to cause a "spurious" increase in the concentration of protein-bound iodine in the plasma (37, 38, 39). This is apparently due to the formation of a non-calorigenic organic compound of iodine. During prolonged treatment with large doses of iodide the unknown compound accumulates very slowly in the plasma, attains a steady concentration during the course of one or two months, and disappears from the body as slowly as it accumulated when the administration of iodide is discontinued. The substance is probably not formed in the thyroid gland since it appeared in the blood stream in two patients with myxedema (38).

In most body tissues conditions are distinctly unfavorable to the oxidation of iodide and its incorporation into an organic molecule. And even if minute traces of iodide are oxidized in certain tissues, one would expect that, as in the thyroid gland, iodinated derivatives of tyrosine would be formed. However, in the acid secretion of the gastric mucosa small quantities of free iodine might easily occur, and could then iodinate some organic compound present in the gastro-intestinal tract. If this hypothetical precursor were a constituent of bile, the iodinated compound formed from it might well undergo enterohepatic circulation which would account for its slow accumulation in the plasma and its equally slow disappearance.

C. *Thiocyanate and Other Anions:* The ability of the thiocyanate ion to inhibit the concentration of the iodide ion by the thyroid gland has been clearly demonstrated by a number of techniques (183, 198, 204). Wyngaarden and his collaborators (209) have recently shown that this property is not peculiar to thiocyanate but is shared by several other anions. In fact perchlorate was ten times as potent as thiocyanate in its ability to prevent iodide accumulation or to discharge iodide which had already accumulated in the thyroid gland. In many respects these anions behave as though they competed with iodide for whatever mechanism is responsible for maintenance of a concentration gradient of iodide ion across the cell membrane. This mechanism is probably an energy-requiring transfer system, although the presence of an iodide-binding protein in the thyroid gland has been postulated as an alternative mechanism (109, 165, 208). It is noteworthy, however, that, in contrast to iodide, only a very small quantity of thiocyanate ion enters the thyroid gland (206, 207), and that even very large concentrations of thiocyanate do not completely abolish the ability

of the thyroid gland to concentrate the iodide ion (140). For further discussion of the possible mode of action of thiocyanate, perchlorate and similar anions, the reader should consult the original papers.

Only a small proportion of patients who are treated for hypertension with thiocyanate develop enlargement of the thyroid gland (10). Furthermore, the goitrogenic action of thiocyanate is rather easily overcome by the simultaneous administration of iodide (6). These observations suggest that the accumulation of a high concentration of iodide ion is not a prerequisite for the manufacture of a normal daily quantity of hormone. When the supply of iodide is generous, the thyroid can obtain the quantity of iodide which it requires even when almost completely deprived of its ability to produce a concentration gradient. However, when the supply of iodide is reduced, the decreased efficiency due to inhibition of the concentrating mechanism becomes a serious handicap. The ultimate consequences are very similar to those of iodine deficiency except that more or less severe hypothyroidism occurs more frequently in patients with goiter due to the administration of thiocyanate.

When the administration of thiocyanate is discontinued, the ability of the thyroid gland to accumulate iodide ion is restored as the thiocyanate is slowly eliminated by the kidneys. As in iodine deficiency (179), the hyperplasia of the thyroid gland decreases rather slowly because of the time needed to reaccumulate an adequate concentration of stored hormone. During recovery the uptake of a tracer dose of radioactive iodine, previously somewhat depressed by the thiocyanate, is often abnormally high (16).

D. *Goitrogenic Drugs of the Thioamide Series:* All of the drugs in this group, which includes thiourea, thiouracil, 2-mercaptoimidazole, and their several derivatives, possess in common an actual or potential —SH group which makes them strong reducing agents and probably accounts for their ability to inhibit the iodination of tyrosine. While there is still doubt concerning their precise mode of action (7, 8, 41, 133), it is well established that adequate doses can produce a practically complete block of thyroid hormone synthesis without in any way altering the ability of the thyroid gland to accumulate iodide ion from the blood stream. The resulting derangement in iodine metabolism is illustrated in Figure 9. In this figure it is assumed that enough blocking agent has been given to the patient of Figure 7 to arrest completely the transfer of iodine from the iodide compartment to the compartment of organic iodine in the thyroid gland, and that this complete block has been maintained for two days. It is also assumed that the thyroid gland has not yet encountered difficulty in mobilizing the large quantity of thyroid hormone necessary to maintain its high rate of secretion. However, it is quite apparent from the figure that if the block of hormone synthesis continues, the delivery of hormone to the blood stream will soon slacken because of approaching exhaustion of hormone previously synthesized and stored in the thyroid. This explains why the response to blocking agents is usually very much more rapid in patients with thyrotoxicosis than in normal subjects. With a complete block, the rapidity of response should theoretically be proportional to K_{GB} which for this subject with thyrotoxicosis is about 12½ times the value

for the normal subject illustrated in Figure 2. However, it should be borne in mind that drugs of the thioamide series may actually hasten the rate at which iodine is lost from the thyroid gland (see Section VI, B, 3). This possibility has been disregarded in the example used here.

Another less obvious effect of blocking agents of this type is also illustrated in Figure 9. The size of the iodide compartment has increased more than six-fold. Two factors contribute to this increase. One is a sudden expansion of the volume of distribution of the iodide ion when the synthesis of thyroid hormone is elim-

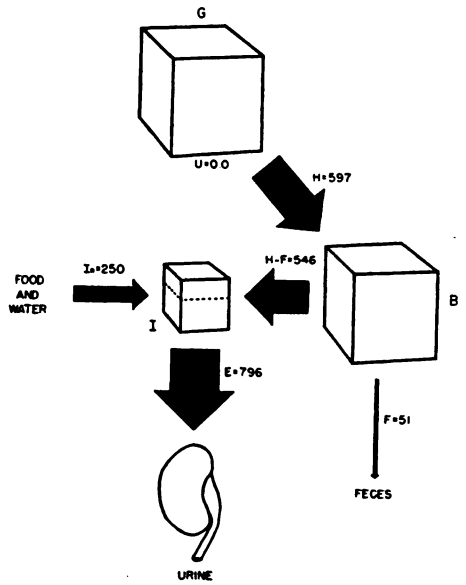

FIG. 9. A diagram of iodine metabolism in the thyrotoxic patient of Figure 7 after two days of complete blockage of thyroid hormone synthesis produced by the administration of a thioamide drug (Table 3, column 7). The construction of the diagram has been explained in the legend of Figure 2.

The portion of the iodide compartment above the dotted line in the figure represents iodide ion within the thyroid gland. The portion below the dotted line represents iodide elsewhere in the body. In addition to the expansion of the iodide compartment, note the enormous increase in the urinary excretion of iodide.

inated. In the unblocked gland the rapid removal of iodide ion for the synthesis of thyroid hormone prevents the actual accumulation of any large quantity of iodide within the gland. During the block, removal no longer occurs and the potential "iodide space" within the gland fills to the capacity determined by the concentration gradient which can be maintained. In the hyperplastic thyroid gland of the rat the concentration gradient may be as high as 250 to 1 (198). In Graves' disease the gradient is probably of the same order of magnitude (28, 183). With a gradient of 250 to 1, the iodide space of a 60 gm. thyroid would therefore be equivalent to 15 liters of the volume of distribution of iodide.

The greater part of the increase in the size of the iodide compartment is not due to the expanded volume of distribution, but rather to the failure of the thy-

roid gland to remove any of the iodide ion from the iodide compartment. Large quantities of iodide continue to enter the compartment from breakdown of hormone in the tissues, and smaller quantities continue to be absorbed from the gastro-intestinal tract. Their removal now depends solely on clearance by the kidneys. Consequently the concentration of iodide rises until the kidneys are able to excrete iodide as rapidly as it is entering. The increased concentration of iodide is so great that it should be easy to detect by chemical methods.

When the synthesis of thyroid hormone is blocked, the rapid entry of radioactive iodine into the thyroid gland should not be mistaken for a true uptake of iodine. Under these circumstances the accumulation of radioactive iodide does not signify any corresponding net *transfer* of stable iodide but simply indicates that the radioactive isotope is attaining distribution equilibrium with the high concentration of stable iodide already present in the gland. Within an hour or two the iodide compartment will have become uniformly labeled and the peak concentration of radioactive iodine in the thyroid gland will have been reached. Thereafter the concentration of radioactive iodide in the thyroid gland will decrease at the same rate as in other segments of the iodide compartment, the rate of decrease depending on the renal clearance of iodide and the volume of distribution of the iodide ion.

If thiocyanate be administered when a large proportion of the tracer dose has entered the thyroid in the form of iodide ion, the sharp decrease in the volume of distribution of iodide due to inhibition of the concentrating mechanism will rapidly reduce the amount of radioactive iodine in the thyroid to about the same concentration as in other tissues. At the same time, the iodide displaced from the thyroid gland must be accommodated by the remainder of the iodide compartment. In consequence the concentration of both stable and radioactive iodide in the blood stream may rise. This was clearly evident in some of the goitrous cretins in whom the block of synthesis of hormone, though apparently a congenital defect, was very similar to the block produced by drugs of the thioamide group (see Section IX, C).

Maintenance of a complete block of hormone synthesis depends upon maintenance of an adequate concentration of drug within the thyroid gland. In clinical practice it is very probable that a complete block is not usually maintained throughout 24 hours, either because the single doses are not sufficiently large or the interval between doses is too long. The net effect upon iodide metabolism is a greater or lesser reduction in the efficiency of hormone synthesis. The clinical response ultimately achieved and the rapidity with which it is attained will then depend not only on how much the efficiency has been impaired, but also on the concentration of iodide ion in the blood perfusing the gland. Unless a reasonably complete blockage of synthesis is produced, the concomitant administration of iodide may slow the response.

XI. DIAGNOSTIC TESTS

The quantitative relationships discussed earlier will now be used to define the factors which influence certain diagnostic tests. In evaluating such tests it

is important to bear in mind that *the clinician is not usually interested in the activity of the thyroid gland, but rather in the action of the thyroid hormone.* Hence the ideal diagnostic test would measure some metabolic effect specifically produced by the hormone in the tissues. Unfortunately no such test is available. While the basal metabolic rate is undoubtedly determined in part by the action of the thyroid hormone, it is also influenced by a wide variety of other factors which are difficult to appraise and impossible to express mathematically. Moreover, the range of variation among euthyroid individuals is very wide. In spite of these well-recognized disadvantages, the basal metabolic rate is still exceedingly useful not only as a diagnostic aid, but also as a means of evaluating the effects of treatment.

The remaining diagnostic tests will be discussed in order of decreasing specificity as measures of the rate of secretion of the thyroid hormone. Although from the theoretical standpoint this will also be the order of decreasing diagnostic value, the practical clinical value of any diagnostic test depends not upon specificity alone but also upon the simplicity and accuracy of the method used. Above all, it depends upon the ability of the test to discriminate between euthyroidism and minimal hyperthyroidism or hypothyroidism. Discriminatory ability can be predicted only in part from a theoretical consideration of the factors which influence a diagnostic test. Clinical utility must finally be established by clinical trial.

A. *The Concentration of Protein-bound Iodine in the Plasma:* The administration of organic compounds of iodine, especially those used as contrast media for roentgenographic diagnosis (97, 148, 154, 169) or the administration of large doses of inorganic iodide (see Section X, B) may cause an elevation of protein-bound iodine in the plasma which bears no relation to thyroid activity. But in the absence of such medication, the concentration of iodine which is bound to the plasma proteins may be taken as a measure of (B), the concentration of hormone in the extrathyroidal compartment. Although the vast majority of the protein-bound material is thyroxine, traces of two other substances have been detected by chromatography (65). One of these has recently been identified as 3:5:3′-l-triiodothyronine by Gross and Pitt-Rivers (66) who also synthesized the compound and showed that it was about three times as potent as l-thyroxine in preventing goiter in rats treated with thiouracil. Because of this surprisingly high potency, Gross and Pitt-Rivers have suggested the possibility that l-triiodothyronine may be the form of the thyroid hormone that is active in the tissues (67).

In Section IX-B it was pointed out that the proportion of the hormone in the tissues which is turned over in unit time probably increases when the concentration of hormone increases. An hypothesis concerning this relationship will now be developed. In so doing, no attempt will be made to distinguish between turnover due to breakdown of hormone in the tissues and turnover due to excretion of hormone. For convenience, therefore, a single rate constant of tissue turnover, K_B, may be defined:

$$K_B = K_{BI} + K_{BF} \qquad 67$$

The simplest possible assumption concerning a relationship between K_B and (B) will now be made, namely that K_B is directly proportional to (B). Hence:

$$K_B = c\,(B) \qquad 68$$

Where c is a proportionality constant with dimensions l^3, m^{-1}, t^{-1}. c may be defined as the liters of compartment B cleared of hormone per hour per microgm. of hormonal iodine present in the compartment. A numerical estimate of c may be obtained by substituting in equation 68 the "typical" values of K_B and (B) given in Table 3 for the normal subject and for the hyperthyroid subject. For the normal subject:

$$0.00222 + 0.00021 = 50\,c$$

$$c = 0.0000486 \text{ liters per hr. per microgm.}$$

For the thyrotoxic subject:

$$0.00676 + 0.00063 = 140\,c$$

$$c = 0.0000528 \text{ liters per hr. per microgm.}$$

The mean of these two estimates of c, 0.0000507, may now be substituted in equation 68:

$$K_B = 0.0000507\,(B) \qquad 68a$$

Combining equations 63 and 64:

$$(B)V_B = \frac{H}{24K_B} \qquad 69$$

Substituting the value for K_B from equation 68a in equation 69:

$$(B)V_B = \frac{H}{0.00122(B)}$$

or

$$(B)^2 = \frac{H}{0.00122V_B} \qquad 70$$

Taking V_B as constant at 24 liters, and substituting this value in equation 70:

$$(B)^2 = 34.1\,H \qquad 71$$

For convenience in testing the validity of equation 71, it may be expressed in logarithmic form:

$$2 \log (B) = \log H + \log 34.1$$

or

$$\log (B) = 0.5 \log H + 0.766 \qquad 72$$

If the assumptions involved in the derivation of equation 72 are reasonable, a plot of log (B) against log H should give a straight line with a slope of 0.5 and an intercept of 0.766.

A truly critical test of the hypothesis just presented would demand simultaneous measurement of the protein-bound iodine and of the daily secretion of hormone in a series of patients with various degrees of hyper- and hypothyroidism. Although methods for estimating the daily secretion of hormone have been developed (see Section VI-B), they have not yet been applied to any such series of patients. However, data concerning the effect of various doses of exogenous hormone on the concentration of protein-bound iodine are available both for hypothyroid patients (201) and for euthyroid subjects (158). These data are presented in Table 4, and plotted in Figure 10. In order to include the data ob-

TABLE 4

The effect of variations in the daily supply of thyroid hormone on the concentration of protein-bound iodine in serum

SUBJECTS	DOSE OF THYROID	MEAN PROTEIN-BOUND IODINE OF SERUM	DAILY SUPPLY OF THYROXINE IODINE		
			Endogenous* (calculated†)	Exogenous‡	H Exogenous plus endogenous
	mgm/day	*microgm./l.*	*microgm/day*	*microgm./day*	*microgm./day*
8 patients with hypothyroidism (201)	0	11	3.5	0	3.5
	65	36	3.5	35.0	38.5
	97.5	45	3.5	52.5	56.0
	130	54	3.5	70	73.5
4 euthyroid subjects (158)	0	53	82.5	0	82.5
	195	66	0	105	105
	390	77	0	210	210
	650	106	0	350	350
	975	128	0	525	525

* It is assumed that, when as much as 195 mgm. of thyroid is given to a euthyroid subject, endogenous production of hormone ceases (59).

† Calculated from the protein-bound iodine of serum by the use of equation 71.

‡ Assuming that 130 mgm. of thyroid by mouth are equivalent to 70 microgm. of endogenous thyroxine iodine (see Section VI-B).

tained before thyroid was administered, equation 71 has been used to calculate the secretion of endogenous hormone from the observed values of (B). By so doing, the initial point in each series is deliberately placed upon the theoretical line. These two points, therefore, contribute no information about the agreement between observation and hypothesis. However, the positions of the remaining points in both series are practically uninfluenced by the endogenous production of hormone. The striking agreement between these experimental points and the theoretical line tends to support the assumption of a linear relationship between K_B and (B).

A further, but less satisfactory, test of this hypothesis depends upon its ability to predict the relationship between the uptake of radioactive iodine and the concentration of protein-bound iodine. Substituting in equation 71 the value

for H from equation 15:

$$(B)^2 = 34.1E\left(\frac{U}{1-U}\right) \qquad 73$$

Skanse (175) has plotted 0.1 (B), the serum protein-bound iodine in microgm. per cent, against 100 (1−U), the per cent of a tracer dose of radioactive iodine excreted in forty-eight hours, for a large series of patients with hyperthyroidism. Since the excretion of stable iodide was not determined, it is necessary to assume

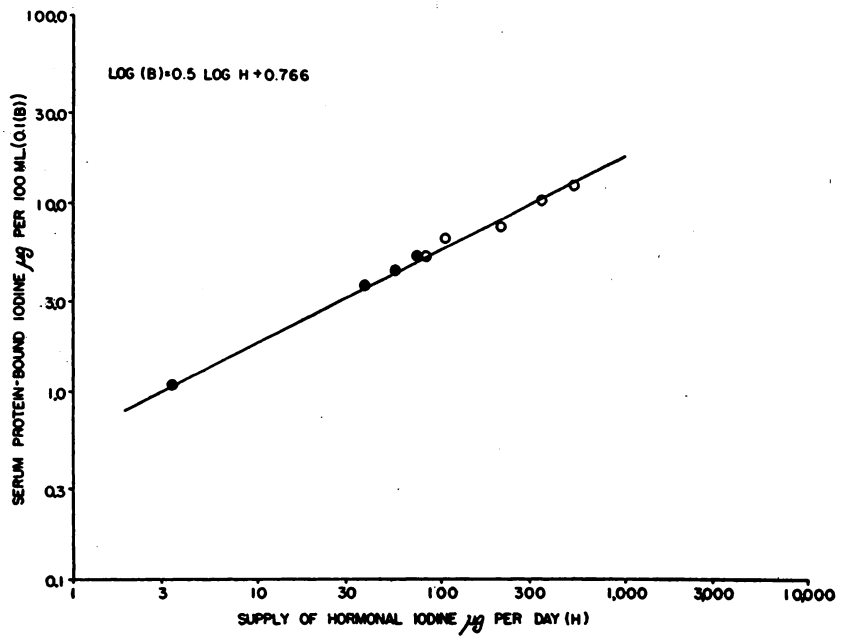

FIG. 10. The relation between the daily supply of thyroid hormone and the serum protein-bound iodine. Both scales are logarithmic. The straight line represents the theoretical relationship defined by equation 72. The calculation of the points is given in Table 4. Solid circles, myxedematous subjects; open circles, euthyroid subjects.

a constant mean value for E which may be taken as approximately 200 (Table 3). Then equation 73 becomes:

$$(B)^2 = 6820\left(\frac{U}{1-U}\right) \qquad 73a$$

The line determined by equation 73a describes fairly well the general trend of the points plotted by Skanse, but the observed excretion of radioactive iodine for any given concentration of protein-bound iodine tends to be somewhat lower than the equation predicts. This is to be expected, since in practice the observed excretion of radioactive iodine is apt to be less than the true value of (1−U) because of incomplete collection of urine.

If equation 73 is used to calculate the values of U corresponding to the normal

limits of (B), (35 to 70 microgm. per liter (154)), the predicted normal range of U for persons with an iodide excretion of 144 microgm. per day is 0.2 to 0.5. This is reasonably close to the normal limits of 0.17 to 0.58 (175) at 48 hours, and 0.10 to 0.40 (200) or 0.17 to 0.43 (81) at 24 hours which have actually been reported by various investigators.

Equation 71 indicates that the *absolute quantity* of thyroxine which is disposed of each day is a function of the second power of the thyroxine concentration. Consequently, if it be assumed that disposal of thyroxine occurs chiefly by the process of breakdown, rather than excretion, this process may be described, superficially at least, as a reaction of the second order. Unfortunately, classification as a second-order reaction does not provide any real insight into the nature of the process itself. One possible interpretation would be that in accelerating metabolic processes in general, thyroxine also accelerates its own utilization. Until more is known about the mechanism of action of the thyroid hormone, further speculations are likely to be fruitless. However, even as an expression of an empirical relationship, equation 71 is useful. In the first place, it helps to explain why patients with myxedema are sensitive, and euthyroid subjects comparatively insensitive, to exogenous thyroid hormone. If x milligrams of thyroid are needed daily to elevate the protein-bound iodine of a patient with complete myxedema from zero to a normal concentration of 50 microgm. per liter of plasma, equation 71 predicts that 4 x milligrams will be required daily to increase the concentration of protein-bound iodine from 50 to 100 microgm. per liter in a euthyroid subject. (It is assumed that in the euthyroid subject the exogenous hormone completely suppresses the secretion of endogenous hormone.) In the second place, equation 71 indicates why the concentration of protein-bound iodine in the plasma seldom, if ever, exceeds 350 microgm. per liter even in very severe hyperthyroidism. To sustain such a concentration, a daily secretion of about 3,600 microgm. of thyroxine iodine would be required. It is scarcely conceivable that the thyroid gland of any patient with a normal intake of iodide could manufacture and secrete hormone more rapidly than this (see Section XI-D). Finally, it is evident from equation 71 why patients with severe hypothyroidism and no detectable accumulation of radioactive iodine in the thyroid region may still have appreciable quantities of protein-bound iodine in the blood stream (see Section IX-A). According to the equation, about one twenty-fifth of the normal rate of secretion of hormone would maintain a concentration of 10 microgm. of protein-bound iodine per liter of plasma, and one one-hundredth of the normal rate of secretion would maintain a concentration of 5 microgm. per liter. These rates of hormone secretion would require uptakes of only about 2 per cent and 0.5 per cent, respectively.

According to equation 70, the concentration of protein-bound iodine in the plasma should depend only upon the rate of secretion of thyroid hormone and the volume of distribution of the hormone in the extrathyroidal tissues. Presumably the volume of distribution varies within a relatively narrow range, more or less in proportion to body weight. Hence the plasma protein-bound iodine may usually be considered a fairly specific index of the rate at which thyroid hormone is secreted by the gland and utilized by the tissues. However,

equation 70 is undoubtedly an oversimplification. In the first place, the constant term, 24 c, is probably not truly constant under all conditions. For example, it may increase during adolescence and decrease in old age (Section VIII-B). It may also increase during exposure to cold, at least in certain species (Section VIII-D). In the second place, in nephrosis the concentration of protein-bound iodine in the *plasma* is low despite compelling evidence that the activity of the thyroid gland is normal or even slightly greater than normal. Recant and Riggs (153) have advanced the hypothesis that a very small quantity of free thyroxine exists in equilibrium with the protein-bound thyroxine of plasma and that it is this minute concentration of unbound hormone which determines the rate of entry of thyroxine into cells. If this theory is correct, a decrease in the thyroxine-binding protein of the plasma, now thought to be α_1 globulin (53), would decrease the concentration of protein-bound iodine needed for maintenance of a *normal* concentration of active free thyroxine in the plasma. Consequently the concentration of hormone *within the cells* in nephrosis would also be normal. If so, the relationship between (B) and H postulated in equation 70 would still be fundamentally correct, except that the ratio of hormone concentration in the plasma to hormone concentration in the cells would be altered, and the plasma protein-bound iodine would therefore not be the usual measure of (B) throughout the whole tissue compartment. This difficulty could presumably be avoided if it were possible to express (B) as the concentration of unbound thyroxine, rather than protein-bound thyroxine, in plasma.

The changes in protein-bound iodine which occur in health and disease have been exhaustively reviewed by Rapport and Curtis (148).

B. *The Uptake of Radioactive Iodine by the Thyroid Gland:* The *theoretical uptake* of radioactive iodine may be defined as the proportion of a tracer dose which would be accumulated by the thyroid in infinite time if there were no secretion of labeled hormone from the gland. The theoretical uptake, herein designated by the symbol U, is a fundamental parameter of thyroid function since it denotes the proportion of the iodide supply which is available for hormone synthesis. In practice, however, it is impossible to measure the theoretical uptake. Some loss of labeled hormone occurs before collection of the administered radioactive iodide is complete, so that the proportion of a tracer dose actually present in the gland at any time after administration is always less than the theoretical uptake. The magnitude of the discrepancy will depend chiefly upon K_{GB}, the ratio of H/24 and Q_G. If serial counts over the gland are available, a fair approximation of the theoretical uptake may be obtained by extrapolating the curve of biological decay back to time zero. The value so obtained may be termed the *extrapolated uptake.*

When the uptake is to be estimated from a single determination of radioactivity in the thyroid, the ideal time for measurement would be the time of *maximum uptake.* However, the time after a tracer dose at which the quantity of radioactive iodine in the thyroid reaches a peak is not constant. In general it will be earlier when K_{GB} is large. Actual measurement of the maximum uptake will therefore also require a series of observations.

The estimate of uptake most commonly used as a diagnostic test may be des-

ignated the *fixed-interval uptake*. A single measurement of radioactivity in the thyroid gland is made at an arbitrarily fixed interval of time, usually 24 or 48 hours, after administration of the tracer dose. Although the 48-hour uptake may be appropriate when K_{GB} is small, it will seriously underestimate the theoretical uptake when turnover of hormone in the thyroid gland is brisk. Indeed, when K_{GB} is very large the radioactivity in the thyroid may already have started to decline at 24 hours. It is evident that if uptake is to be estimated from a single measurement at a fixed interval of time, a compromise must be reached between

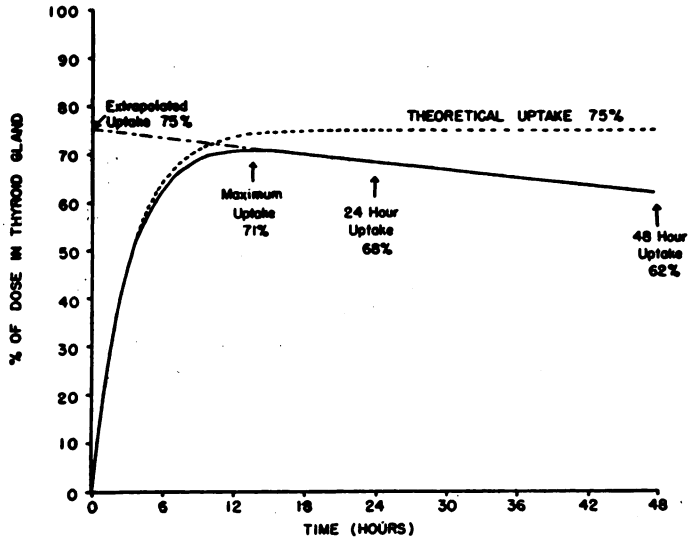

FIG. 11. The relationship between various estimates of the uptake of radioactive iodine. The solid line represents the theoretical curve for the uptake and early decay of radioactive iodine in the thyroid gland after intravenous administration of a tracer dose at time zero to the thyrotoxic subject of Figure 7. The broken line represents the manner in which radioactive iodine would accumulate were there no loss of labeled hormone from the gland. The line of long and short dashes represents an extrapolation of the decay curve to time zero. The various measures of uptake illustrated in this figure are discussed in the text.

an interval so short that collection by a sluggish gland will be grossly incomplete, and an interval so long that a considerable quantity of labeled hormone will have been secreted by an active gland.

The relationship between these various estimates of uptake and the theoretical uptake for the hyperthyroid patient of Figure 7 and Table 3 is illustrated in Figure 11.

Not illustrated is still another method of estimating the uptake, namely, measurement of the proportion of a tracer dose excreted in the urine. Subtraction of this proportion from unity gives the *uptake by difference*. Theoretically, this method has much in its favor since radioactive iodine can be measured more accurately in urine than in the thyroid gland. Furthermore, even if a considerable quantity of labeled hormone is secreted, comparatively little of it will be

metabolized to iodide during the period of urine collection (usually 48 hours). But in practice, this method is apt to overestimate the theoretical uptake because of failure to obtain complete collections of urine. The uptake by difference will also be unduly high if the clearance of iodide by the kidneys is much reduced.

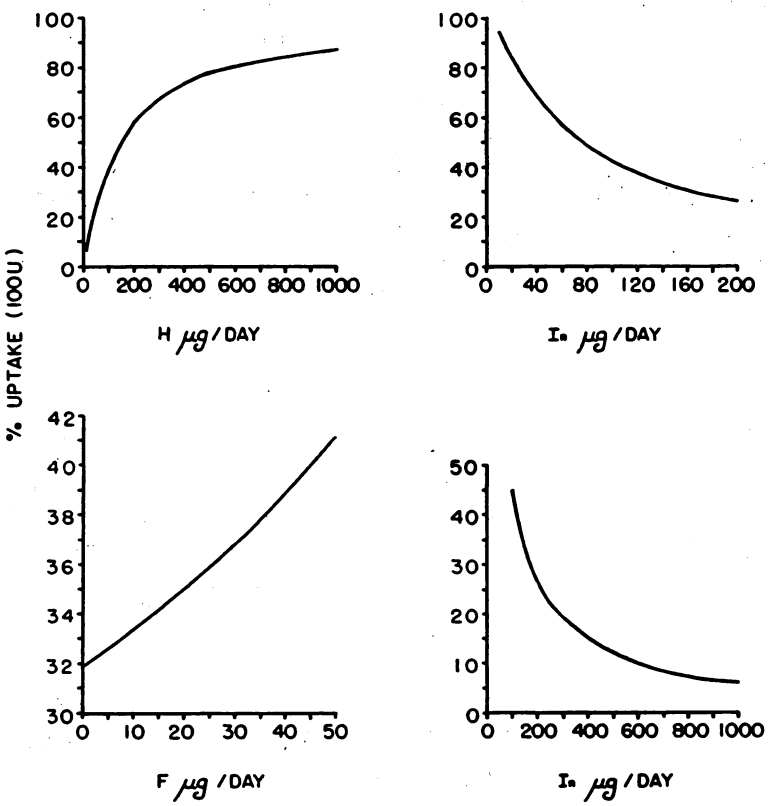

FIG. 12. The influence of variations in H, F and In upon the uptake of radioactive iodine. The curves have been calculated from equation 74. For each graph, only the factor plotted on the abscissa is allowed to vary, the other two factors retaining the normal values listed in Table 3, column 3.

The factors influencing the uptake may be defined by solving equation 13 for U:

$$U = \frac{H}{H + In - F} \qquad 74$$

The uptake is therefore not solely dependent upon the rate of secretion of the thyroid hormone but is also profoundly influenced by the intake of iodide and by the excretion of organic iodine. However, unlike the diagnostic tests to be discussed subsequently, the uptake is unaffected by the renal clearance of iodide. In Figure 12 are illustrated the changes in uptake which are produced

when H, In and F are allowed to vary over ranges which may actually be encountered. It is well established that a reduction in iodide intake will eventually lead to an increase in uptake (Section VIII-F) and that the administration of large doses of iodide will practically abolish the uptake (Section X-B). However, it is apparently not widely appreciated that a modest increase in the daily supply of iodide will alter the uptake very perceptibly. For example, the continued daily ingestion of as little as five grams of iodized salt containing iodide in a concentration of one part in ten thousand would reduce the uptake of the normal subject of Figure 2 from 33 per cent to 10 per cent, and of the hyperthyroid subject of Figure 7 from 75 per cent to 46 per cent.

C. *The Clearance of Iodide by the Thyroid Gland:* Myant and his collaborators (121, 123) and Keating's group (81, 82) have rightly proposed the thyroid clearance as a direct measure of thyroid activity. But, as an index of hormone secretion, the thyroid clearance is even less specific than the uptake. The factors which influence the thyroid clearance are given by equation 6:

$$C_G = \frac{C_K H}{In - F} \qquad 6$$

It is evident that the thyroid clearance of iodide is determined in part by the renal clearance of iodide, as well as by the factors which influence uptake.

The importance of the renal clearance of iodide as a factor influencing the thyroid clearance is well illustrated by the data of McConahey *et al.* (105). In 9 euthyroid subjects with normal renal function the mean renal clearance of iodide was 33.3 ml. of plasma per minute and the mean "extra-renal clearance" (practically equivalent to the thyroid clearance) was 26.3 ml. of plasma per minute. In 12 euthyroid subjects with impaired renal function the corresponding mean values were 8.7 and 6.5 ml. of plasma per minute, respectively. In contrast, the mean *uptake* calculated by equation 38 from these figures was 0.44 for the subjects with normal kidneys and 0.43 for the subjects with renal impairment.

D. *The Rate of Accumulation of Iodide by the Thyroid Gland:* Keating and his collaborators have employed the rate constant of accumulation, K_{IG}, as a diagnostic test (82). The factors which determine K_{IG} will now be derived. At equilibrium, by definition:

$$K_{IG} = \frac{H}{24Q_I} \qquad 75$$

Substituting in equation 75 the value for Q_I from equation 44,

$$K_{IG} = \frac{H}{24V_I(I)} \qquad 76$$

Substituting in equation 76 the value for (I) from equation 5a

$$K_{IG} = \frac{0.06C_K H}{V_I(In - F)} \qquad 77$$

K_{IG} is therefore slightly less specific than C_G as a measure of H, since it is in-

fluenced by the volume of distribution of iodide as well as by the renal clearance, the intake and the excretion of organic iodine.

If C_K, V_I, In and F remain essentially constant, both the thyroid clearance and the accumulation rate have one theoretical advantage over the uptake. They are *directly* proportional to H and consequently undergo large changes

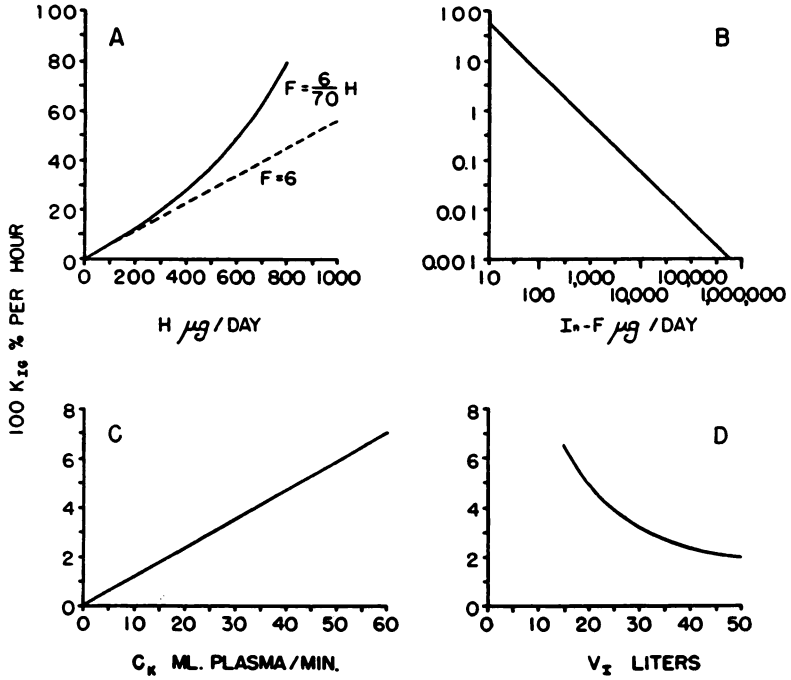

FIG. 13. The influence of variations in H, In-F, C_K and V_I upon K_{IG}, the rate constant of iodine uptake by the thyroid gland, expressed as per cent of the quantity of iodide present in compartment I which is accumulated per hour. The curves have been calculated from equation 77. Except for the solid line in graph A, only the factor plotted on the abscissa is allowed to vary in a given graph. The other factors retain the normal values listed in Table 3, column 3. In the solid curve of graph A, F is taken as 0.0857 H. In curves A, C and D, both scales are arithmetic. In curve B, both scales are logarithmic. Curves A, B and C may also be used to illustrate the influence of the various factors on C_G, the thyroid plasma clearance of iodide, provided that the numerals on the ordinate are multiplied by 4.16. For example, in curve C when the renal clearance is 10 ml. of plasma per minute the rate of accumulation is 1.17 per cent per hour and the thyroid clearance would therefore be 4.85 ml. of plasma per minute.

throughout the hyperthyroid range. But in equation 74 for the uptake, H appears in both numerator and denominator. As H increases, therefore, the rate of change of U becomes smaller and smaller. For example, if H increases from 150 microgm. per day to 300 microgm. per day (corresponding roughly to a change from high normal to distinctly hyperthyroid), U would only increase from 0.51 to 0.68. In contrast C_G would increase from 35 ml. per minute to 70 ml. per minute, and K_{IG} from 0.083 per hour to 0.167 per hour.

In Figure 13 are illustrated the changes in K_{IG}, expressed as per cent per

hour, which would be produced by variations in H, In-F, C_K and V_I. The first three curves will serve equally well for the thyroid clearance provided the ordinates are multiplied by 4.16 to convert them to a scale of ml. of plasma per minute.

A point of considerable importance is illustrated by Figure 13, part A. The dotted line indicates that if F remained constant despite an increasing rate of secretion of hormone, the accumulation rate would be directly proportional to H. It is much more probable that F actually increases with H as indicated by the solid line. If so, the excretion of organic iodine may well become a very important limiting factor as the rate of secretion of hormone rises. While an increase in iodide intake due to polyphagia may for a time offset the increase in the excretion of organic iodine, it can scarcely continue to do so indefinitely. As the gap between In and F narrows, In-F rapidly becomes smaller and smaller, and K_{IG} must become larger and larger. The same argument obviously applies to the uptake and to the thyroid clearance. In section XI-A it was suggested that the rate of secretion of hormone in hyperthyroidism is seldom, if ever, greater than 3,600 microgm. of thyroxine iodine per day. If 6/70 of this were excreted in organic form, the intake of iodide would obviously have to exceed 309 microgm. per day. And since it is unlikely that the clearance of iodide by the thyroid can be much greater than 1000 ml. of plasma per minute, the daily intake of iodide would probably have to be at least 429 microgm. per day, almost three times the normal intake. This argument clearly implies that in patients with severe hyperthyroidism, improvement would be produced by limiting the daily intake of iodide. While this is of theoretical, rather than therapeutic interest, it is quite possible that some of the "spontaneous" exacerbations and remissions so characteristic of untreated hyperthyroidism may be due to unrecognized fluctuations in the daily intake of iodide.

Astwood (182) has developed a purely empirical measure of accumulation which he has termed the *accumulation gradient*. It may be defined as the slope of the straight line which is usually obtained when the radioactivity in the thyroid gland, expressed as counts per second, is plotted on the ordinate against the square root of the time in minutes on the abscissa. When so defined, the numerical value of the accumulation gradient depends not only upon the rate of accumulation but also upon the dose of radioactive iodine and the method of counting. It would seem preferable to plot the radioactivity in per cent of the administered dose (see Figure 14) so that values from different laboratories could be compared. However, this would not obviate the fundamental objection, clearly recognized by Astwood himself (141), that the accumulation gradient does not measure any parameter of thyroid function which can be defined mathematically. It is therefore impossible to derive any equation which will express the factors influencing the accumulation gradient. Indeed, the relationship between radioactivity in the gland and square root of time is at best only approximately linear, and then only for a variable portion of the curve of accumulation (Figure 14). However, Astwood points out that in spite of its limitations the accumulation gradient is a convenient means of estimating, from a

few early observations, the general trend of accumulation, and he has used it to good advantage in studies of the potency and duration of action of antithyroid drugs (182).

E. *The Conversion Ratio:* Several investigators have used diagnostic tests which depend upon the rate at which labeled hormone appears in the blood stream after a tracer dose of radioactive iodine (29, 48, 72, 171, 172, 173). Usually a sample of blood is collected at some fixed interval of time after the administration of the tracer and the total radioactivity in the plasma is measured. The

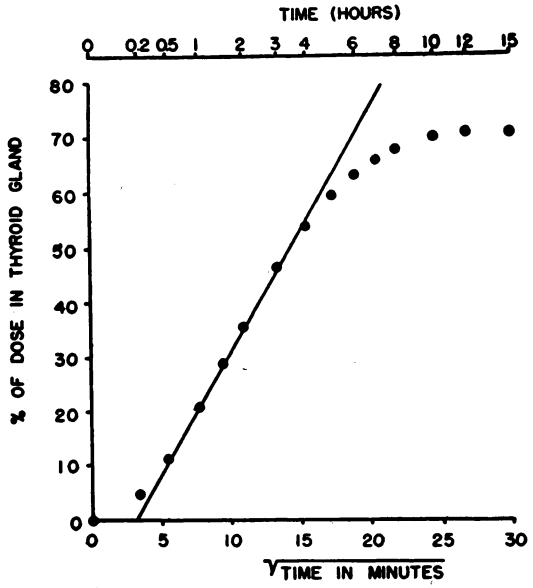

FIG. 14. The theoretical accumulation of radioactive iodine by the thyroid gland of the thyrotoxic subject of Figure 7 (solid circles) plotted against the square root of the time in minutes. For comparison, the time in hours is given on the upper scale.

When plotted in this manner, the curve of uptake is a skewed sigmoid curve. The straight line, whose slope is proportional to the accumulation gradient, fits a portion of the points reasonably well but it is evident from the figure that the exact slope of the line will depend upon which of the points the investigator arbitrarily chooses to disregard.

plasma proteins are then precipitated and the radioactivity in the precipitate is measured as an index of the concentration of labeled hormone. The proportion of the total radioactivity which is protein-bound is termed the conversion ratio, C.R. Hence, by definition:

$$\text{C.R.} = \frac{(\text{B})_t^*}{(\text{I})_t^* + (\text{B})_t^*} \qquad 78$$

A rigorous mathematical analysis of the factors affecting the conversion ratio would require simultaneous solution of equations 41, 42 and 43. The difficulties inherent in this have already been mentioned (Section VII). However, the interval between administration of the radioactive iodine and determination of

the conversion ratio is usually only 24 hours, and it is probably safe to assume that during this time very little radioactive iodide derived from breakdown of labeled hormone in the tissues is entering the iodide compartment. This simplifying assumption makes it possible to derive a reasonably accurate expression for the factors which influence the conversion ratio.

$(I)^*_t$, the concentration of radioactive iodide at any time, t, after the administration of a tracer dose may be calculated from equation 46:

$$Q^*_{It} = Q^*_{I_0} e^{-(K_{IE}+K_{IG})t} \qquad 46$$

But since the tracer is assumed to be instantaneously absorbed and distributed throughout the iodide compartment:

$$Q^*_{I_0} = 1 \qquad 79$$

Also, for convenience, define:

$$K_{IE} + K_{IG} = K_I \qquad 80$$

Substituting these values in equation 46:

$$Q^*_{It} = e^{-K_I t} \qquad 81$$

Substituting in equation 81 the value for Q_I from equation 44:

$$(I)^*_t V_I = e^{-K_I t} \qquad 82$$

Solving for $(I)^*_t$:

$$(I)^*_t = \frac{e^{-K_I t}}{V_I} \qquad 83$$

To obtain an expression for $(B)^*_t$, an equation must first be derived for Q^*_{Gt}, the quantity of radioactivity in the thyroid gland at any time, t, after the administration of a tracer dose. Since it is assumed that within the period now under consideration all of the radioactivity is either in compartments I and G or has been excreted in the urine:

$$Q^*_{It} + Q^*_{Gt} + Q^*_{Et} = 1$$

or

$$Q^*_{It} = 1 - Q^*_{Gt} - Q^*_{Et} \qquad 84$$

Where Q^*_{Et} is the quantity which has been excreted from time zero to time t. By definition:

$$U = \frac{Q^*_{G_t}}{Q^*_{G_t} + Q^*_{E_t}} \qquad 85$$

Solving for Q^*_{Et}

$$Q^*_{E_t} = Q^*_{G_t}\left(\frac{1}{U} - 1\right) \qquad 86$$

Substituting this value for Q^*_{Et} in equation 84:

$$Q^*_{I_t} = 1 - Q^*_{G_t} - \frac{Q^*_{G_t}}{U} + Q^*_{G_t} = 1 - \frac{Q^*_{G_t}}{U} \qquad 87$$

Solving for Q^*_{Gt}

$$Q^*_{Gt} = U\,(1 - Q^*_{It}) \qquad 88$$

Substituting in equation 88 the value for Q^*_{It} from equation 81:

$$Q^*_{Gt} = U\,(1 - e^{-K_I t}) \qquad 89$$

Since the assumption is being made that no breakdown or excretion of radioactive hormone occurs during the period under consideration, equation 43 reduces to:

$$\frac{dQ^*_B}{dt} = K_{GB}\,Q^*_G \qquad 43a$$

Integrating:

$$\int_0^{Q^*_{Bt}} dQ^*_B = \int_0^t Q^*_{G_t}\,K_{GB}\,dt \qquad 90$$

Substituting in equation 90, the value for Q^*_{Gt} from equation 89:

$$\int_0^{Q^*_{Bt}} dQ^*_B = \int_0^t U(1 - e^{-K_I t})K_{GB}\,dt \qquad 91$$

Solving for Q^*_{Bt}:

$$Q^*_{B_t} = UK_{GB}\,t + UK_{GB}\left(\frac{1}{K_I}\right)(e^{-K_I t} - e^{-K_I 0})$$

or

$$Q^*_{B_t} = UK_{GB}\left[t + \frac{1}{K_I}(e^{-K_I t} - 1)\right]$$

or,

$$Q^*_{B_t} = UK_{GB}\left[t - \frac{1}{K_I}(1 - e^{-K_I t})\right] \qquad 92$$

Substituting in equation 92, the value of Q^*_{Bt} from equation 63 and solving for $(B)^*_t$:

$$(B)^*_t V_B = UK_{GB}\left[t - \frac{1}{K_I}(1 - e^{-K_I t})\right]$$

$$(B)^*_t = \frac{UK_{GB}\left[t - \frac{1}{K_I}(1 - e^{-K_I t})\right]}{V_B} \qquad 93$$

The value of $(B)^*_t$ from equation 93 and the value of $(I)^*_t$ from equation 83 may now be substituted in equation 78:

$$\text{C.R.} = \frac{\frac{UK_{GB}}{V_B}\left[t - \frac{1}{K_I}(1 - e^{-K_I t})\right]}{\frac{e^{-K_I t}}{V_I} + \frac{UK_{GB}}{V_B}\left[t - \frac{1}{K_I}(1 - e^{-K_I t})\right]} \qquad 94$$

However, equation 94 still contains several terms which are not primary factors. These must now be eliminated. Solving equation 58 for K_{GB}:

$$K_{GB} = \frac{H}{24Q_G} \qquad 58a$$

Multiplying equation 58a by equation 74:

$$UK_{GB} = \frac{H^2}{24Q_G(H + In - F)} \qquad 95$$

Solving equation 9 for K_{IE}

$$K_{IE} = \frac{0.06C_K}{V_I} \qquad 96$$

Substituting this value for K_{IE}, and the value for K_{IG} from equation 77 in equation 80:

$$K_I = \frac{0.06C_K}{V_I} + \frac{0.06C_K}{V_I}\left(\frac{H}{In - F}\right)$$

or

$$K_I = \frac{0.06C_K}{V_I}\left(1 + \frac{H}{In - F}\right) \qquad 97$$

Substituting in equation 94 the value for UK_{GB} from equation 95, and the value for K_I from equation 97:

$$C.R. = \frac{\left(\frac{H^2}{24Q_G V_B(H + In - F)}\right)\left[t - \frac{1 - e^{-\frac{0.06C_K t}{V_I}\left(1 + \frac{H}{In-F}\right)}}{\frac{0.06C_K}{V_I}\left(1 + \frac{H}{In - F}\right)}\right]}{\left[\frac{e^{-\frac{0.06C_K t}{V_I}\left(1 + \frac{H}{In-F}\right)}}{V_I}\right] + \left(\frac{H^2}{24Q_G V_B(H + In - F)}\right)\left[t - \frac{1 - e^{-\frac{0.06C_K t}{V_I}\left(1 + \frac{H}{In-F}\right)}}{\frac{0.06C_K}{V_I}\left(1 + \frac{H}{In - F}\right)}\right]} \qquad 98$$

Even from this approximate equation it is obvious that the conversion ratio is an exceedingly complex function, dependent upon no fewer than eight variables: H, Q_G, V_I, V_B, In, F, C_K and t. Indeed at first glance it might seem quite useless as a diagnostic test. Yet an analysis of how its component parts influence the conversion ratio demonstrates that under normal circumstances it may possess great discriminatory power. The numerator, representing $(B)^*_t$, consists of two main parts. The part enclosed in parenthesis *increases* very rapidly as the rate of secretion of thyroid hormone increases because it is strongly influenced by its numerator, H^2. The part enclosed in brackets also increases with H, but very much more slowly. At the same time the term in the denominator which represents $(I)^*_t$ *decreases* very rapidly because, as H increases, the nega-

tive exponent of e becomes larger. The net result is that the conversion ratio is (theoretically) almost zero when H is low, rises very rapidly while H is increasing over a small range, and very quickly approaches its maximum value of 1. In Figure 15 the conversion ratio calculated from equation 98 has been plotted against the plasma protein-bound iodine, 0.1 (B), as calculated from H using equation 71. The values for the other variables are those listed for the normal subject in Table 3, and they have been taken as constant throughout. The time is 24 hours. Note that the conversion ratio describes a very steep sigmoid curve as the concentration of protein-bound iodine in the plasma rises from 4.0 to 12.0 microgm. per cent. Its mid-point, the point of maximum discriminatory power, is close to 8.0 microgm. per cent which is usually considered the upper limit of normal. This fortunate circumstance means that, so long as the variables other than H do not differ much from the ones chosen for illustration, the conversion

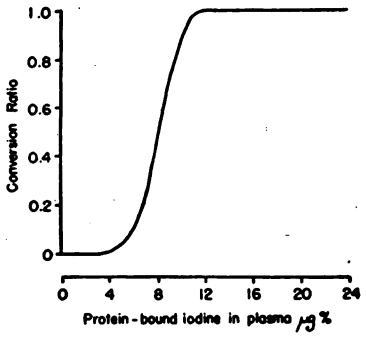

FIG. 15. The conversion ratio, calculated from equation 98 for various values of H, plotted against the concentration of protein-bound iodine in the plasma, calculated from equation 71 for the same values of H. The factors other than H which influence the conversion ratio retain the normal values listed in Table 3, column 3.

ratio calculated at 24 hours should theoretically distinguish rather sharply between euthyroidism and hyperthyroidism. At the same time it would be useless for the diagnosis of hypothyroidism. Since the sigmoid curve will be shifted to the left if the time interval is increased, a longer time interval might be appropriate for hypothyroidism but would probably be useless for the diagnosis of hyperthyroidism.

Unfortunately, one can never be sure that in a given patient the values of such factors as Q_G, In-F and C_K are actually close to the ones chosen for illustration. Furthermore, accurate measurement of $(B)_t^*$ and $(I)_t^*$ requires the administration of a dose of radioactive iodine too large to be employed legitimately as a tracer for routine diagnostic studies. At present, therefore, the conversion ratio must be interpreted with considerable caution, and does not seem likely to displace the simpler diagnostic tests in clinical practice.

XII. CONCLUSION

It is perhaps appropriate that this review has ended with a consideration of the conversion ratio. *Observation* of the conversion ratio requires only the sim-

plest chemical preparation of a sample of plasma, followed by two determinations of radioactivity. Its *interpretation* has required a rather complicated mathematical analysis, and even this has had to be based upon certain simplifying assumptions not fully in accord with biological facts. It is easy for observation to outdistance precise interpretation.

Yet there is reason to be satisfied with the progress which has already been made towards an orderly arrangement of the observed facts into a solid foundation of quantitative theory. With so much to build upon, further progress should be rapid. In the reviewer's opinion, future advances are likely to stem chiefly from the simultaneous use of several techniques in the detailed investigation of small groups of patients. In particular, he would urge that studies with radioactive iodine be supplemented by the determination of stable iodine not only in blood but also in urine. Many of the present uncertainties concerning quantitative aspects of iodine metabolism in man might thereby be resolved.

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